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SUPERNUMERARY CHROMOSOMES IN ZEA MAYS[1]

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INTRODUCTION

Zea mays has been found to have a haploid chromosome number varying between 10 and 15 and corresponding variations in the somatic number.

Kuwada (*13*, *14*, *15*, *16*),[2] the pioneer investigator of chromosomes in maize, abandoned in 1919 the possibility that 12-chromosomed sweet varieties of Zea represented a primitive type—a fact overlooked by the writer in previous articles.

The view that 10 is the primitive chromosome number in maize has been supported in recent articles by Longley (*17*, *18*), Kiesselbach and Petersen (*11*), Fisk (*9*, *10*), Reeves (*22*), and Randolph (*21*).

The writer investigated several old and well-established varieties of maize, all of which had uniformly 10 chromosomes, before finding some with supernumerary chromosomes. Plants with a somatic number of more than 20 chromosomes occur occasionally in the varieties Golden Bantam and Stowell's Evergreen and more frequently in Country Gentleman, Black Mexican, White Sheath, a strain of Pawnee flour corn, and in a highly inbred strain of White Dent (Crosby). The present paper reports on the character and behavior of extra chromosomes based on a three-year study of chromosomes in microspore mother cells in *Zea mays*.

MATERIAL AND METHOD

The plants for this study of maize were grown at the Arlington Experiment Farm, Rosslyn, Va., and in the department's greenhouses in Washington, D. C. Many varieties and strains were examined, but the six above-named varieties or strains were the only ones in which plants with a haploid number of more than 10 were found.

The determination of chromosome numbers was confined to microspore mother cells. To make certain that haploid numbers found in mother-cell material were constant for individual plants, preparations were made from the major tassel and from the tassels of suckers of the same plant. Except for occasional abnormal-appearing cells, all parts of a plant gave the same chromosome number. Consequently, after trying this test on many plants of several varieties without finding serious discrepancies, the writer felt assured that by determining the chromosome number from microspore mother cells of a

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[2] Reference is made by number (italic) to "Literature cited," page 783.

plant one could satisfactorily ascertain the chromosome number of individual plants.

The method used in the laboratory of making cytological preparations of mother-cell material has passed through an evolutionary stage during the last three years. Very early it was found that the microspore mother-cell material of Zea lends itself very satisfactorily to the use of iron-aceto-carmine as a killing and staining method. This method, however, necessitated the use of fresh material and so restricted study to the short period when corn was in flower.

To overcome this difficulty and to lengthen the period of study, material was killed in 70 per cent alcohol, and it was found that with this poorly balanced killing fluid the material was sufficiently well preserved to be useful in making iron-aceto-carmine preparations even after the material had been stored in alcohol for months.

The next step was to substitute acetic-absolute (1 to 3) killing fluid for 70 per cent alcohol. The specimens are left in this fluid 20 minutes and then washed with absolute alcohol. Thompson (*29*) has also described this method of preserving material for future study when stained with iron-acetocarmine. It was found that excellent preparations could be made from material killed in this manner. The mother cells were not as pliable as those stained from fresh material, but by careful staining they could be kept very much longer without deterioration. In fact, the deterioration of slides was generally due to a drying out of the staining liquid. This results in a concentration of the stain to the point of crystallization, giving particles of carmine that may be confusing.

A modification of Mann's (*20*) method for making permanent mounts has recently been tried and found completely satisfactory. Slides are cleaned, a light layer of fixative applied, and then anther material previously killed in acetic-absolute solution is macerated in a drop of water on the slide. The microspore mother cells are teased out, and with the edge of a cover glass the anther wall fragments are removed, leaving the mother cells floating in water. The slide is now dried in the same manner as a slide covered with paraffin sections. The mother cells seem to withstand the treatment quite as satisfactorily as those embedded in paraffin. After the slide has dried for an hour or more it can be stained, dehydrated, and mounted in balsam in the usual manner.

Carmine-stained preparations have some advantages over material stained with haematoxylin. The chromosomes are semitransparent when stained with carmine, and the strands making up an individual can be traced better than when stained with haematoxylin. Small grains found in the nuclei of some preparations, which resemble chromatin fragments when stained with haematoxylin, fail to take a carmine stain, and so this confusion is overcome by using the latter stain.

Slides are studied by the aid of a one-sixteenth objective and × 8, × 15, and × 18 oculars. The drawings used in the figures were made with a camera lucida, magnification 1,500.

CHROMOSOME CHARACTERISTICS

A detailed study of the chromosomes of *Zea mays* at diakinesis made it apparent that chromosome individuality was distinguishable. Fisk (*10*), in her recent article, has opened the way by describing some

of the types which bivalent chromosomes assume. These shapes are illustrated in Plate XI, Figure 34, of her article.

The bivalent chromosomes at diakinesis in their partially contracted condition are sufficiently constant in shape to allow a comparison of individual chromosomes in the same nucleus as well as in different nuclei. Just prior to the fading out of the nucleolus, eight of the chromosomes are simple rings or rings with the two ends overlapping. Six of these generally appear as rings, although at times they are not completely closed. The chromosomes A, B, E, F, G, and H of Fisk's (*10*) drawing seem to correspond to these six rings. Two are rings with a pronounced overlapping of the two ends and possibly correspond to chromosomes C and I of her diagram.

Of the 10 chromosomes the 2 remaining ones are more characteristic. One is large and often appears as a figure 8, which Fisk (*10*) describes as "larger and slightly more twisted." The tenth is a small chromosome and has no easily described shape.

Figure 1, A–F, shows a few illustrations in which all 10 chromosomes are at a stage when their characteristic shapes can be best recognized. B shows clearly the two ring-shaped chromosomes with overlapping ends and the figure 8 chromosome, but two of the six ring-shaped chromosomes are very open and are hardly recognizable.

Figure 2, A and B, has been arranged to show the constancy of the chromosome shapes in four different microspore mother cells of the same plant as well as in two related plants. The chromosomes have been arranged according to the scheme apparent in Fisk's drawing (*10, pl. XI, fig. 34*). The minor variations apparent in the attempted comparison are either partially or wholly due to the usual difficulties experienced in finding cells in the same stage of development, chromosomes in the same stage of contraction, and chromosomes in a favorable plane for showing their characteristic shapes.

Figure 3, A–F, shows figures from mother cells with more than 10 chromosomes. The more outstanding chromosome shapes can be identified. Each set shows the eight open or closed ring-shaped chromosomes and the figure-8 chromosome. The supernumerary chromosomes are distinguishable from any of these two classes, but frequently resemble so closely the small tenth chromosome of the normal set that identification becomes uncertain. In some prophase figures the two units of an extra chromosome are very loosely held together, and occasionally they are separate and appear as two univalent chromosomes. A and B show a pair of univalent chromosomes. B and F are drawings of chromosomes from plants with odd somatic number which consequently show an unpaired univalent present. Univalents generally can be recognized by their small size and compact globular shape.

Figure 2, C, is a continuation of the chromosome comparison of A and B. Each of the four sets illustrated shows extra chromosomes at the end of the series. The first has five supernumerary chromosomes, all approximately the same size and resembling most nearly the smallest of the 10 normal chromosomes. Their shapes are in no way characteristic and for this reason are not confused with the ring-shaped and the figure-8 chromosomes.

This study of chromosome shapes in *Zea mays* has been confined to the first reduction division of the microspore mother cell. Chromosomes at the metaphase stage (fig. 4) assume shapes which give some indications of the point of spindle fiber attachment. Eight

chromosomes have central or subterminal points of fiber attachments. These eight seem to correspond with the eight ring-shaped chromosomes. Those rings with overlapping ends seem to correspond in number with those that have subterminal but not central points of

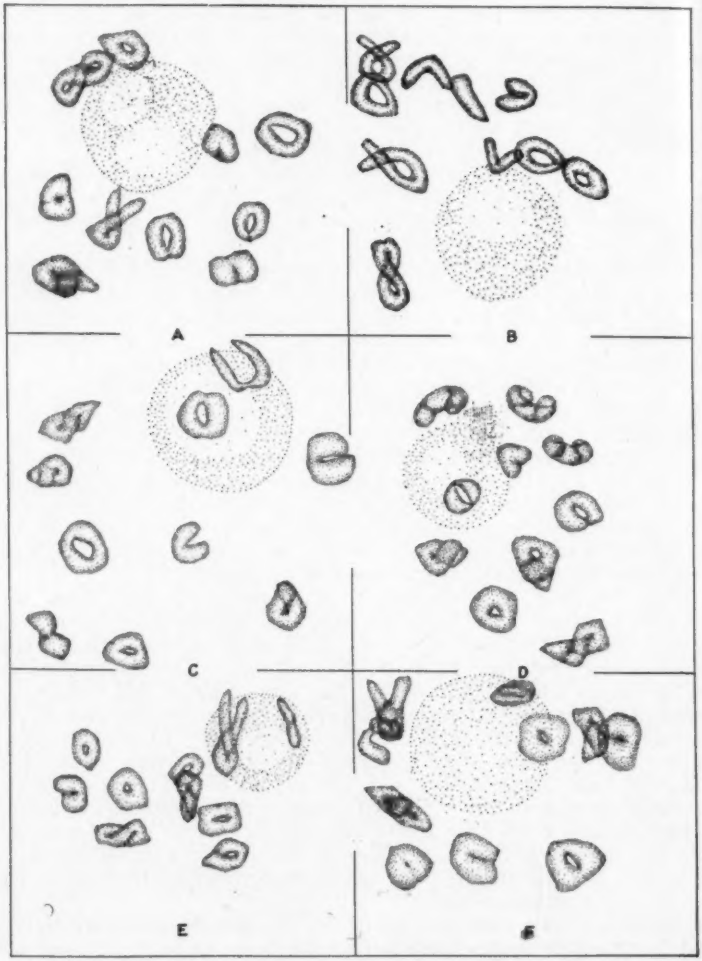

FIG. 1.—Diakinesis in the microspore mother cells of *Zea mays*, showing characteristic shapes assumed by the 10 bivalent chromosomes: A, Golden Bantam sweet corn; B, Black Mexican sweet corn; C, Red × Crosby; D, Wichita flint; E, Hybrid Ah-645; F, Country Gentleman sweet corn

fiber attachments, while the more nearly perfect ring-shaped chromosomes have central fiber attachments. The two remaining chromosomes have approximately terminal points of fiber attachment. One, a large chromosome, corresponds to the figure-8 chromosome of

diakinesis, and the other, the smallest of all chromosomes, corresponds to the tenth small chromosome already referred to.

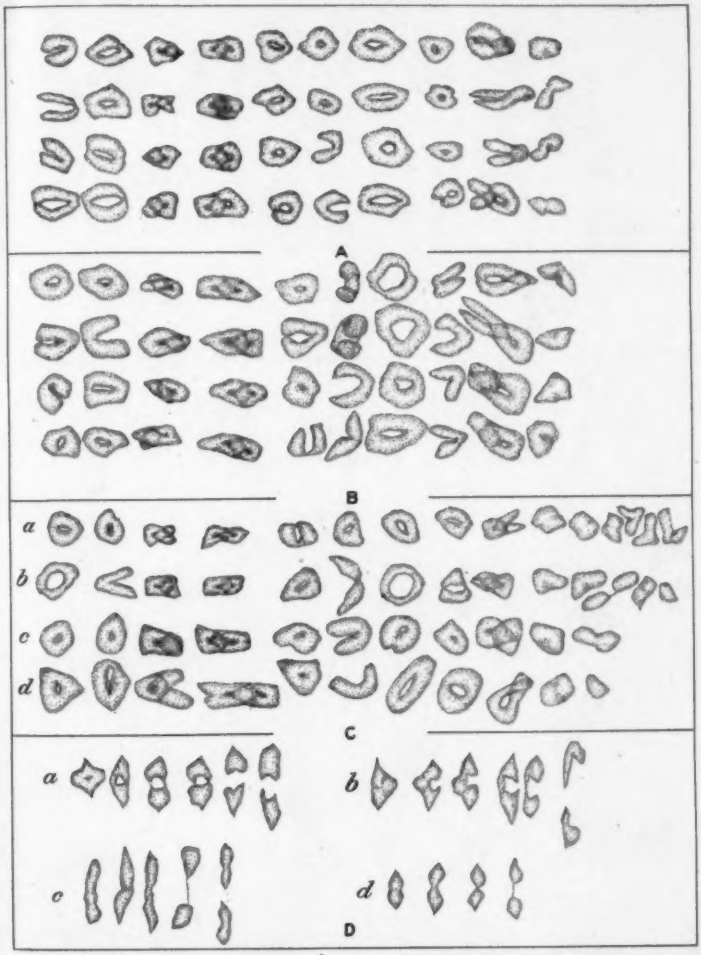

FIG. 2.—Bivalent chromosomes of maize: A, Four chromosome sets from hybrid Ah-645-1748, arranged to show the similarity of chromosome shapes in four microspore mother cells of the same plant; B, four chromosome sets from hybrid Ah-645-1751; C, *a*, a chromosome set from a plant of Black Mexican sweet corn with 15 bivalent chromosomes; *b*, a similar set from a sister plant with a univalent and 13 bivalent chromosomes; *c*, a set from a third sister plant with 11 bivalent chromosomes; *d*, a chromosome set from Country Gentleman sweet corn with a univalent and 10 bivalent chromosomes; D, chromosomes from heterotypic metaphase plates of microspore mother cells, showing the region of fiber attachment: *a*, Stages in the division of chromosomes with central fiber attachment; *b*, stages in the division of chromosomes with subterminal but no central fiber attachment; *c*, a larger chromosome with terminal fiber attachment; *d*, a smaller chromosome with terminal fiber attachment

Figure 2, D, illustrates the differences apparent in chromosomes during their separation at the heterotypic division. Large chromosomes with central spindle fiber attachments split first at the middle.

The final points of separation are the two extremities (D, *a*). When the fiber is attached subterminally but not at the center, the split starts

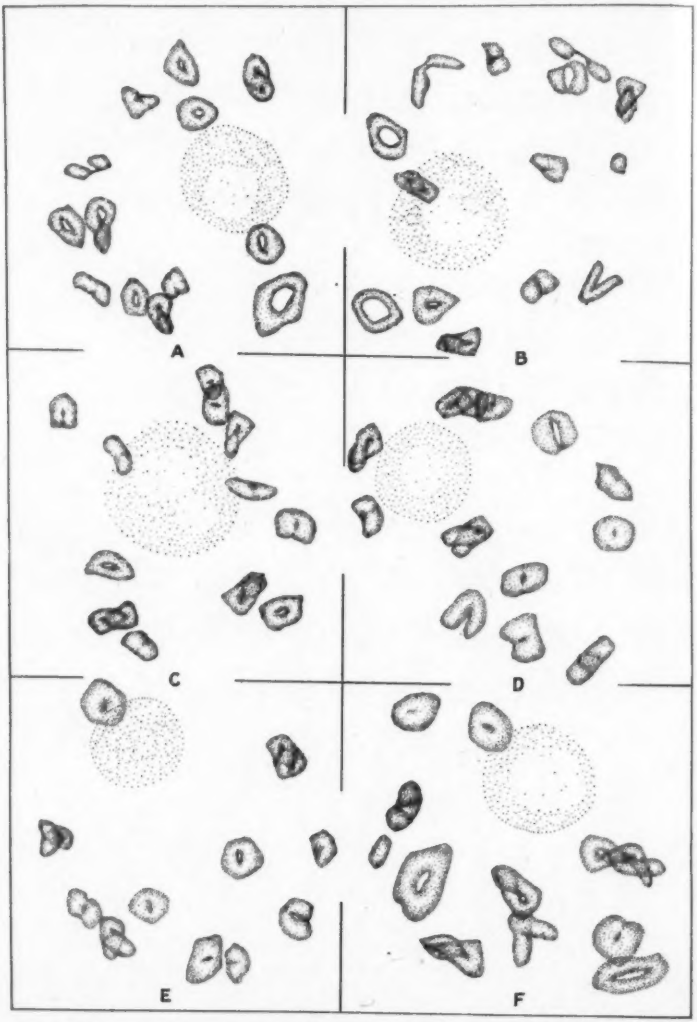

FIG. 3.—Diakinesis in the microspore mother cells of *Zea mays*, showing the characteristic shape of both the normal and the supernumerary chromosomes: A, Black Mexican sweet corn, showing 13 bivalent chromosomes; B, Black Mexican sweet corn, showing a univalent and 13 bivalent chromosomes; C, Black Mexican sweet corn, ×Dwarf (F^1), showing 12 bivalent chromosomes; D, Black Mexican sweet corn, showing 12 bivalent chromosomes; E, Black Mexican sweet corn, showing 11 bivalent chromosomes; F, Golden Bantam sweet corn, showing a univalent and 10 bivalent chromosomes

toward one end, and at the final stage the two units are held together at one extremity only (D, *b*). The two chromosomes with terminal fiber attachments are best distinguished by their size and rodlike

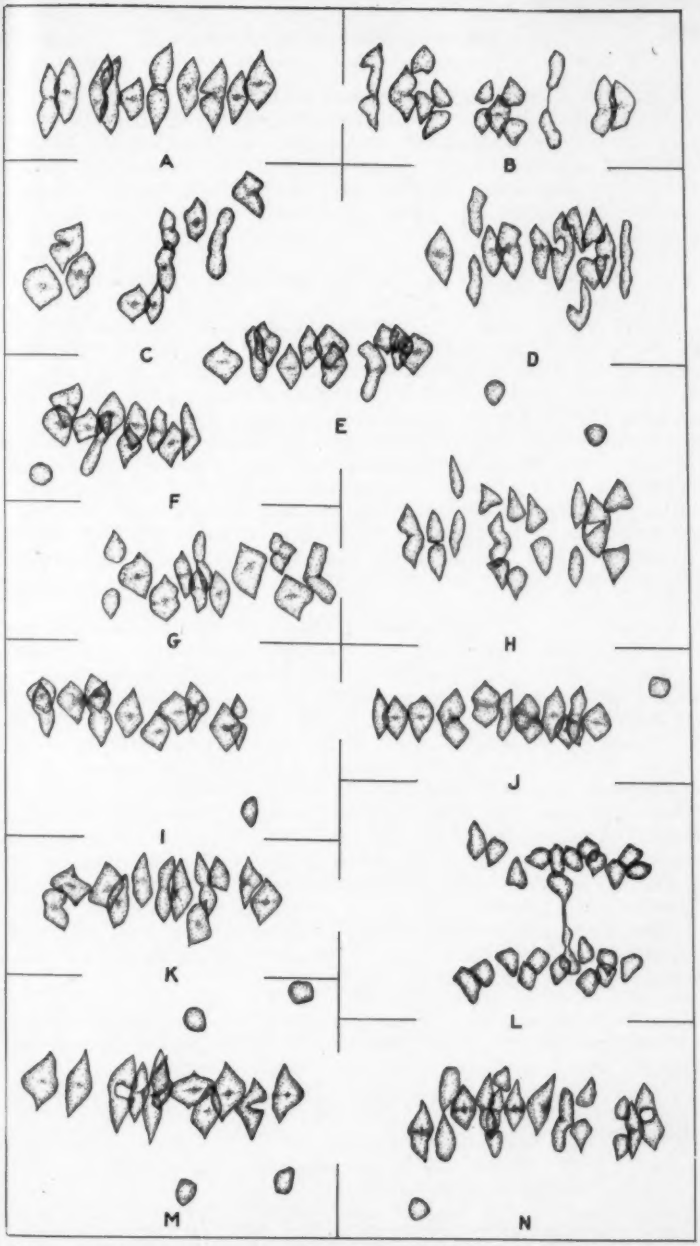

FIG. 4.—Heterotypic metaphases from microspore mother cells of *Zea*, showing the shape and behavior of chromosomes at this phase: A and C, Country Gentleman sweet corn with 10 bivalent chromosomes; B and D, Black Mexican sweet corn with 10 bivalent chromosomes; E and G, Country Gentleman sweet corn with a univalent and 10 bivalent chromosomes; F, Country Gentleman sweet corn with 11 bivalent chromosomes; H, Hybrid Ph-285 with 2 unpaired and 10 paired chromosomes; I, Country Gentleman sweet corn with a univalent and 11 bivalent chromosomes; J, Red×Crosby (F_6) with a univalent and 11 bivalent chromosomes; K, Red×Crosby with 12 bivalent chromosomes; L, Black Mexican sweet corn (anaphase) with 12 divided chromosomes; M, Black Mexican sweet corn with 4 unpaired and 11 paired chromosomes; N, Black Mexican sweet corn with a univalent and 13 bivalent chromosomes

shape. The large one is shown in D, *c*, the smaller in D, *d*. Fisk (*10*) finds one small chromosome that occasionally divides early, a behavior that has been noticed in the tenth small chromosome and for supernumerary chromosomes. Consequently, supernumerary chromosomes are classified with the small chromosome of the normal 10, due to similarity in size, shape, and mode of division at the heterotypic metaphase.

BEHAVIOR OF SUPERNUMERARY CHROMOSOMES

Before attempting to describe the behavior of supernumerary chromosomes during meiosis, it seems in order to describe the normal behavior of chromosomes in plants with the basic number 20. Fisk (*10, Table 1*), reports that in progenies usually characterized by 20 somatic chromosomes some cases in which more or less than 10 chromosomes possibly are present at diakinesis were found, and only one case of nondisjunction in the homotypic division which gave rise to pollen cells with 9 and 11 chromosomes. Only infrequently has the writer found such irregularities in the number and behavior of chromosomes at diakinesis in microspore mother cells of plants with 20 chromosomes. It is likely that through such irregularities in chromosome distribution plants with more than 20 chromosomes have arisen.

A study of the offspring from plants with 20 chromosomes grown at Arlington in 1926 failed to give any plants with more than the normal number. Some of these were descendants of plants carrying several supernumerary chromosomes, but evidently the grandparents did not carry factors that affected the distribution of the 10 basic chromosomes, since none of the F_2 plants had more than 20 chromosomes. A similar study of 21 progenies in 1924 gave one exception—six plants were grown from a Black Mexican parent supposed to have 20 chromosomes. Five of these plants had 22 chromosomes and one had 21. Unfortunately, all the cytological material from the parent was used in making the determination, thus preventing a confirmation of this chromosome count. Consequently, there was no means of determining whether an error in observation had been made or whether these plants with extra chromosomes were the offspring of a plant with 20 chromosomes. The chromosome number of the six plants indicates the former.

TABLE 1.—*Distribution by chromosome number of 97 plants, both parents having 20 chromosomes, some of the grandparents having more than 20 chromosomes*

Progeny designation	Designation of grandparents		Designation of parents		Chromosome number of grandparents		Chromosome number of parents		Chromosome classes of progeny	
	Female	Male	Female	Male	Female	Male	Female	Male	20	20+
P-2	(a)	(a)	532	532	------	------	20	20	29	0
P-83-1	C-1	C-1	89-B_4	89-B_3	20	20	20	20	14	0
P-83-2	C-1	C-1	89-B_3	89-B_2	20	20	20	20	7	0
P-83-3	D-5	D-5	94-A_{13}	94-A_{20}	21	21	20	20	25	0
P-83-4	G-4	B-4	97-A_6	97-A_3	24?	21	20	20	22	0

[a] Commercial seed.

The figures given in Table 1, taken from material collected in 1925, indicate that the 20 constantly present chromosomes of maize are regular in their behavior at meiosis. Exceptions similar to that found the previous year must be of rare occurrence. Consequently, in this study of the inheritance of a supernumerary chromosome, irregularities in the distribution of the 20 chromosomes of the normal set are regarded as insignificant.

The data contained in Table 2 are in marked contrast with those in Table 1. When the parent plant has 22 instead of 20 chromosomes, F_1 plants with from 20 to 24 chromosomes are found instead of 22, the number which would be found if the eleventh chromosome behaved as regularly during the reduction division as the 10 normal chromosomes.

TABLE 2.—*Distribution by chromosome number of 73 F_1 plants, both female and male parents having 22 chromosomes*

Progeny designation	Designation of parent plants		Chromosome number of parents		Chromosome classes of F_1 offspring				
	Female	Male	Female	Male	20	21	22	23	24
P-2-1	8035	8035	11	11		3	1	1	
P-2-2	8067	8067	11	11			3	2	5
P-2-3	8226	8226	11	11	1	1			
P-2-4	8284	8284	11	11	5	2	1	1	
P-83-1	G-4	G-4	11	11		2	2		
Ah-848-1	556B	556B	11	11		10	5	25	
Ah-848-2	280-B_{18}	280-B_{22}	11	11		1	1	1	
Total					6	19	13	30	5

In order to analyze the apparent irregularities in the behavior of supernumerary chromosomes, the simplest case was chosen. Plants with 21 chromosomes were crossed reciprocally with plants with 20 chromosomes and with each other, by which process three groups of progenies were obtained—those in which the female parent carried one extra chromosome, those in which the male parent carried the extra chromosome, and finally those in which both parents carried an extra chromosome. The chromosome number was determined in a large number of these F_1 plants. Tables 3, 4, and 5 show the number of plants obtained in the various chromosome classes of these three related groups.

In the five progenies given in Table 3 only the male parent had an extra chromosome. The presence of a few plants with 22 chromosomes in four of the five progenies studied shows that the distribution of the extra chromosome at the time of pollen formation in the male parent was irregular. Regular division of a supernumerary chromosome in either the heterotypic or the homotypic division in the microspore mother cell of the male parent would give in these crosses equal numbers of 20 and 21 chromosomed plants. This is clearly not the case, for in four of the five progenies studied the number of 20-chromosomed plants was much in excess of those with 21 chromosomes, and there is present a third class of plants with 22 chromosomes. It seems unlikely that a differential death rate has been effective in eliminating pollen grains with extra chromo-

somes, since in progeny P–83–2 the 11-chromosomed pollen, effective in bringing about fertilization, outnumbers the 10-chromosomed grains, and in progeny P–2–2 there is one 12-chromosomed pollen grain to three 10-chromosomed grains. The ratio in the latter is that expected when the extra chromosome nondisjuncts in the homotypic division.

TABLE 3.—*Distribution by chromosome number of 112 F_1 plants, the female parent having 20 and the male parent 21 chromosomes*

Progeny designation	Designation of parent plants		Chromosome number of parents		Chromosome classes of F_1 offspring			Percentage of nondisjunction
	Female	Male	Female	Male	20	21	22	
P–2–1	C_3	B_1	20	21	10	2	2	69.5
P–2–2	64–B_3	64–B_4	20	21	24	0	8	[a] 100.0
P–4–1	508–3	508–4	20	21	23	11	4	42.1
P–83–1	94–B_2	94–B_3	20	21	13	0	1	[a] 100.0
P–83–2	95–A_7	95–A_{17}	20	21	6	9	0	[a] 0

[a] Calculated from the ratio of the two observed classes.

Table 4 shows a similar behavior for this extra chromosome when present in the megaspore mother cell. There are many more 20-chromosomed plants than 21-chromosomed plants in all progenies studied, and again there are a few 22-chromosomed plants. These facts indicate an irregular behavior of the extra chromosome at meiosis of the megaspore mother cell of 21-chromosomed plants similar to that noticed in the microspore mother cell. In the production of an embryo-sac mother cell, however, three of the four cells of the linear tetrad die. All four of the progeny ratios of Table 4 show a deficiency in 12-chromosomed cells, indicating that both a differential death rate, which eliminates some of the cells with extra chromosomes, and nondisjunction have been effective in distorting the observed ratios between the three chromosome groups.

TABLE 4.—*Distribution by chromosome number of 170 F_1 plants, the female parent having 21 chromosomes and the male parent 20 chromosomes*

Progeny designation	Designation of parent plants		Chromosome number of parents		Chromosome classes of F_1 offspring			Percentage of nondisjunction
	Female	Male	Female	Male	20	21	22	
P–2–1	64–B_4	64–B_3	21	20	19	11	0	[a] 26.70
P–83–1	93–B_{13}	93–B_{15}	21	20	27	12	1	36.00
P–83–2	94–B_7	94–B_1	21	20	31	21	2	24.40
P–83–3	95–A_6	95–A_{16}	21	20	32	14	0	[a] 39.1
Total					109	58	3	26.8

[a] Calculated from the ratio of the two observed classes.

Table 5 gives different classes of plants resulting from a union of pollen and eggs, both produced from plants with 21 chromosomes. A regular behavior of the supernumerary chromosomes would give plants with the chromosome numbers 20, 21, and 22, in the proportion 1 : 2 : 1. A glance at the observed populations shows that not only

is the ratio of 20, 21, and 22 chromosomed plants much distorted but also that a fourth class with 23 chromosomes has appeared.

These additional data, therefore, confirm the assumption that an extra chromosome is erratic in its behavior at meiosis of both the megaspore and the microspore mother cell.

TABLE 5.—*Distribution by chromosome number of 274 F_1 plants, both female and male parents having 21 chromosomes*

Progeny designation	Designation of parent plants		Chromosome number of parents		Chromosome classes of F_1 offspring				Percentage of nondisjunction	
	Female	Male	Female	Male	20	21	22	23	Female *	Male
P-2-1	50-B_3	50-B_4	21	21	14	5	3	3	26.8	100
P-2-2	69-B_{10}	69-B_4	21	21	26	14	19	6	26.8	100
P-83-1	95-A_{17}	95-A_{18}	21	21	12	19	13	0	26.8	0
P-83-2	96-A_{12}	96-A_{12}	21	21	20	20	4	2	26.8	35
P-83-3	97-A_{11}	97-A_{11}	21	21	16	11	10	2	26.8	65
P-83-4	97-A_{17}	97-A_9	21	21	17	22	6	1	26.8	10

* Value taken from Table 4.

Cytological observations of microspore mother cells with 21/2 chromosomes have shown that the extra univalent does not divide in the first of the reduction divisions. This observation is substantiated by the study of F_1 progenies one or both parents of which had 21 chromosomes. If this extra chromosome divided and the halves went to opposite poles in the first reduction division, there would be produced pollen and eggs with 10 and 11 chromosomes only. This can not be the case since in the F_1 progenies are found plants that must have originated from pollen and eggs with 12 chromosomes. Frequently in microspore mother-cell preparations with an extra chromosome, one of the chromosomes is found nondisjuncting in the homotypic division. It is this behavior, combined with the failure of a supernumerary chromosome to divide in the heterotypic division, that produces some pollen and eggs with 12 chromosomes. There is thus provided a reasonable explanation of the irregular chromosome behavior that brings about the aberrant ratios found in this hybrid offspring.

Figure 5, A and B, is a schematic drawing which shows the sequence of events in the production of cells with extra chromosomes in the linear and in the pollen tetrads.

The figures given in Tables 3 and 4 show that nondisjunction has significantly modified the expected ratios of the progenies studied. A more critical consideration of these aberrant ratios made it apparent that the amount of nondisjunction for individual progenies may be very definitely estimated. For example, progeny P–4–1, Table 3, has 23 plants with 20 chromosomes, 11 with 21 chromosomes, and 4 with 22 chromosomes. The excess of the first over the second class is 12 plants. This excess added to the 4 plants of the third class, gives 16 plants out of a total of 38 that, within the limits of random sampling, must have originated through nondisjunction. There was in this progeny, therefore, 42.1 per cent of nondisjunction.

When possible, the percentages of nondisjunction were calculated by Fisher's formula (*8, p. 25*) for determining the maximum likelihood. The percentages of nondisjunction thus calculated are given in the last column of each table.

Table 3 records the behavior of an extra chromosome in microspore mother cells. The percentages vary from 0 to 100, while those for the megaspore mother cell, recorded in Table 4, vary only from 20 to 36. These percentages suggest that the extra chromosome when present in the less-protected microspore mother cell is more erratic in its behavior than when present in the well-protected megaspore mother cell. The individual behavior of the five progenies listed in Table 3 are so different that they can not rightly be classed

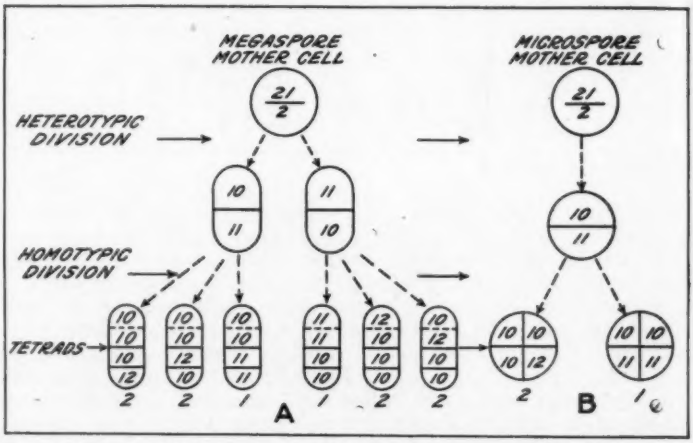

FIG. 5.—Diagram showing distribution at meiosis of 21/2 chromosomes to the four cells of a tetrad: A, Megaspore mother cells. The first division gives two cells, one with 10 and the other with 11 chromosomes. The second division gives six types of tetrads, two (see A–1) in which the division of the eleventh chromosome is normal and four (see A–2) in which there is nondisjunction of the two halves of the eleventh chromosome; B, microspore mother cells. The first division gives two cells, one with 10 and the other with 11 chromosomes. The second division gives two types of tetrads, one (see B–1) in which the division of the eleventh chromosome is normal and one (see B–2) in which there is nondisjunction of the two halves of the eleventh chromosome

together, while the four progenies listed in Table 4 are not significantly different, which permits their combination into one group and the calculation of the total percentage of nondisjunction for the combined group. For the megaspore mother-cell material studied this percentage is 26.8, while the percentage for the microspore mother-cell material is variable, ranging from 0 to 100 per cent.

Progenies P–2–1, listed in Table 4, and P–2–2, listed in Table 3, may be used for comparing the behavior of this extra chromosome in the megaspore and microspore mother cells of the same plant. The percentage of nondisjunction for the former is 26.62, while that for the latter is 100. Compared by Fisher's (*8, p. 84*) X^2 test of Independence, these two progenies give an X^2 of 10.106+, indicating that there exists a significant difference in the reciprocal cross.

DISCUSSION

Variations in the form and size of chromosomes have frequently been referred to and pictured by cytological investigators. Sharp (*23, p. 173*) discusses individual chromosome characteristics, and his simile comparing a chromosome set to a series of unlike wheels in a watch seems very apt. Just such a set of 10 chromosomes and apparent differences in anatomical characteristics of sister chromosomes are to be found in maize.

Carothers (*5*) in Circotettix, Taylor (*24, 25, 26, 27, 28*) in Fritillaria, Abstroemeria, and other genera, and Ferguson (*7*) in Aloinae have recently investigated anatomical chromosome characteristics. One outstanding character found constant in chromosomes is the region of fiber attachment and the associated constriction. Taylor (*28*) considers the heterotypic division unfavorable for detecting constrictions. However, the region of the fiber attachment shows distinctly at the heterotypic metaphase of microspore mother cells of *Zea mays*. This character is constant for each chromosome and serves as a useful mark for chromosome identification. In all diploid forms of maize there are constantly two chromosomes with approximately terminal, two with subterminal, and six with central regions of fiber attachment.

Shapes of chromosomes, such as rods, V's, and J's, for somatic chromosomes and double V's or rings, double J's, X's and 8's for bivalent chromosomes, are described by Belling (*2, 3*) for hyacinths. These characteristic shapes are not peculiar to hyacinths, but are observed by cytologists in innumerable plant forms. When seen at diakinesis in a favorable plane the bivalent chromosomes of *Zea mays* can be classified as six rings or double V's, two loops with overlapping ends or double J's, and a figure 8.

Size of chromosomes is also a much-used characteristic for identifying individual chromosomes and even whole chromosome sets. Babcock and Lesley (*1*) find similarity of chromosome size is a useful criterion of relationship of Crepis species. Outstanding size differences are apparent in Zea chromosomes. The six rings have appreciable size differences, the figure-8 chromosome is large, and one chromosome of the normal set is most readily identified by its small size.

The determination of definite chromosome characteristics for each of the 10 chromosomes making up the set of all diploid forms of maize should make it easier to identify and trace the behavior of extra chromosomes in aneuploid forms. Supernumerary chromosomes in maize have terminal points of fiber attachment, are small, and in general resemble closely the tenth small chromosome of the normal set.

In order to determine whether supernumerary chromosomes are new bodies or simple duplications of chromosomes of the normal set, recourse may be had to genetic analysis. Such an analysis is now in progress.

The present study is concerned with the inheritance of a single extra chromosome. Carother's (*5*) article on the distribution of chromosomes in Circotettix has demonstrated that the laws of heredity apply to the segregation of chromosomes making up a normal set. Belling (*2*) has shown that in a triploid hyacinth the

chromosomes in excess of the diploid number are distributed by chance. Watkins (*30, 31*) and Kihara (*12*) have studied the behavior of unpaired chromosomes in Triticum hybrids, and have found that although they are distributed at meiosis by the law of chance, there is a differential death rate eliminating pollen with extra chromosomes.

These observations of *Zea mays* indicate that one extra chromosome in a megaspore or a microspore mother cell is affected by some factor that modifies the chance distribution. The different possibilities have been tested, and the conclusion reached that nondisjunction of the extra chromosome in the homotypic division causes the production of some pollen and eggs with 12 chromosomes. It has been found that the percentage of nondisjunction for a particular progeny can be determined approximately from the ratios between 10, 11, and 12 chromosomed gametes. These percentages show that nondisjunction of an extra chromosome in the second division of the microspore mother cells in the five progenies studied is variable, ranging from 0 to 100 per cent, while in the four progenies of megaspore mother cells nondisjunction was observed to occur in approximately 26 per cent of the cells carrying an extra chromosome.

Outstanding examples of plants in which pollen grains with a certain chromosome number are more effective in bringing about fertilization than other grains with a lower or a higher number are Triticum, already referred to; *Oenothera lata*, in which Lutz (*19*) believes that only male gametes with seven chromosomes function; the Globe mutant of Datura, found by Buchholz and Blakeslee (*4*) to transmit the extra chromosome in about 3 per cent of the pollen grains; and *Nicotiana tabacum*, in which Clausen and Goodspeed (*6*) observed a similar elimination of pollen carrying an extra chromosome. In maize there seems to be no indication that male gametes with extra chromosomes are less effective in bringing about fertilization than those having the normal set of 10. In the selection of an embryo-sac mother cell from the linear tetrad, however, there are definite indications of a differential death rate which increases the number of 10-chromosomed female gametes at the expense of those carrying 12 chromosomes.

These data on the distribution of more than one supernumerary chromosome are too incomplete to be included in the present article.

SUMMARY

Ten has been found to be the haploid chromosome number present in most maize varieties and forms. These 10 normal chromosomes, when viewed in the heterotypic division, have individual characteristics such as size, shape, and position of fiber attachment. This chromosome set is composed of six double V's, two double J's, a figure 8, and a small chromosome with no easily describable shape.

The behavior of the normal chromosome set during the reduction phases is, with rare exceptions, regular.

A few maize forms have been found in which, frequently or infrequently, plants appear with supernumerary chromosomes.

Extra chromosomes resemble in size and shape the smallest of the normal chromosome set.

The distribution of a supernumerary chromosome to the four daughter cells has been studied and found to be erratic.

It goes undivided to one or the other of the poles in the first reduction division, and nondisjunction occurs in varying percentages in the second division.

In microspore mother cells nondisjunction varies from 0 to 100 per cent in the five progenies studied.

In megaspore mother cells nondisjunction is a more stable factor and is found to be approximately 26 per cent in the four progenies studied.

Pollen viability seems to be in no way associated with chromosome number. On the other hand, a differential death rate seems to eliminate part of the embryo-sac mother cells with two supernumerary chromosomes.

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DEVELOPMENT OF THREE MIDSEASON VARIETIES OF CABBAGE RESISTANT TO YELLOWS (FUSARIUM CONGLUTINANS WOLL.)[1]

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INTRODUCTION

The nature and increasing destructiveness of cabbage yellows (*Fusarium conglutinans* Woll.) throughout the central belt of States from coast to coast have already been amply discussed in previous reports (*2*, *3*, *4*).[4] It is now 15 years or more since the improvement of cabbage varieties with respect to their resistance to this disease was begun by Jones and his associates. The first efforts with the late-storage type resulted in the development of Wisconsin Hollander (*2*), while selections from two standard sauerkraut varieties have led to the introduction of Wisconsin All Seasons and Wisconsin Brunswick (*3*). These three varieties are now in common use where the yellows disease prevails and where the needs are met by these types. In each case they are late-maturing varieties eminently suited for the production of late storage cabbage on the one hand and a late sauerkraut crop on the other.

In many sections where yellows occurs, the early and midseason crops equal or exceed the late crop. It is therefore natural that with the increasing importance of the disease a keen demand has arisen for resistant strains maturing earlier than those in use and conforming in type to the somewhat varied demands. Accordingly, as already noted (*4*), selection work was begun some years ago from a number of the earlier varieties, there being now included in the list All Head Early, Glory of Enkhuizen, Copenhagen Market, Jersey Wakefield, and Charleston Wakefield.

Progress with the first three varieties named has reached the point where certain of the selections not only possess high degrees of resistance to the yellows organism, but also have reasonable uniformity in other respects such as time of maturity, shape and quality of head, color, and productiveness. It has therefore been found expedient to multiply the seed of these lines and to introduce them to the trade channels, where it is expected that they will fill a place as midseason varieties in yellows-infested regions. These new strains resemble in their main features the varieties from which they were selected. They possess the added character of resistance and some variations as to type, and these differences seem to warrant varietal distinction. The names of the new varieties which will be

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[4] Reference is made by number (italic) to "Literature cited," p. 806.

used in the present discussion and which are recommended for general usage are listed below along with the names of the parent varieties from which they were derived.

Yellows-resistant variety	*Parent variety*
All Head Select.	All Head Early.
Globe.	Glory of Enkhuizen.
Marion Market.	Copenhagen Market (midseason strain.)

The purpose of this paper is to describe the methods used in the development of the new varieties and to record the salient details of the experimental work which have yielded the present results.

PRINCIPLES OF CABBAGE BREEDING INVOLVED

In cabbage the blossoms are open-pollinated and as a rule are cross-fertilized by insects. The writers' experience with artificial self-pollination of many cabbage seed plants has shown that in the main they are only slightly self-fertile, but sufficiently so to produce enough seed for pure-line selections. In cases where certain strains have been repeatedly selfed for three generations, some reduction in size of plants has been noted. Thus, continued pure-line selection may have some disadvantage where the stimulation of heterosis seems to be essential to productiveness. As a result the usual method employed for the improvement or maintenance of a standard variety of cabbage is first to select a number of individuals possessing certain desirable characters. These are reset the following season in an isolated plantation where cross-pollination between individuals may occur. By planting the seed of each plant separately the third season, individuals from the best of these may be selected and the process repeated. Thus there is attained a gradual approach toward homozygosity for certain characters, while vigor is maintained by allowing the plants from selected rows to cross-pollinate.

This method of selection has been followed with unusual success in the work of Jones and his associates (*2*, *3*) in the development of the three Fusarium-resistant varieties already mentioned, Wisconsin Hollander, Wisconsin All Seasons, and Wisconsin Brunswick. Although in the commercial varieties from which these strains were selected only an occasional plant showed resistance to the disease, the progenies of such individuals were highly resistant in the second or third generation. Strains completely free from susceptible plants were never attained, however, by this method.

More recent work (*8*) indicates that resistance is dominant over susceptibility. Moreover, a study of the F_2 generation of crosses between highly resistant and highly susceptible parents shows that segregation occurs at a ratio very close to 3 resistant to 1 susceptible, and therefore indicates that resistance and susceptibility are allelomorphic unit characters (*8*). One would therefore expect to encounter difficulty in completely eliminating the recessive character of susceptibility by means of the head-to-row selection from open-pollinated plants.

In the development of the three resistant late varieties already mentioned (*2*, *3*) some marked deviations in type have resulted. This is most striking in the Wisconsin Hollander, which was selected

from two individuals of Ferry's Danish Ballhead variety. The latter is a late-maturing storage type which produces a spherical, compact head with comparatively few outer leaves and a short stem. The Wisconsin Hollander, by way of contrast, matures some two weeks later than Ferry's Danish Ballhead and produces a taller, ranker plant with a much larger head which is elliptical rather than spherical in vertical cross section. The Wisconsin All Seasons matures somewhat later than the standard All Seasons and in spite of repeated head-to-row selection still varies considerably as to shape of head, leaf color, and uniformity in maturing. One of the chief reasons for these deviations probably lies in the fact that the disease reduced the plants available for the original selections to a very small group within which the opportunity to select for conformity to the standard type was decidedly limited. This change was so marked in the initial step with Wisconsin Hollander that it has seemingly been impossible so far to bring the resistant strain to the original type of the standard commercial variety through head-to-row selection from individuals allowed to open-pollinate.

The deviations in type which obtain in Wisconsin Hollander, Wisconsin All Seasons, and Wisconsin Brunswick are fortunately not so great as to impair their value in meeting certain commercial needs. The problem before the writers has been to produce supplementary resistant varieties earlier in maturity and of various types. Selections from the earlier standard varieties, according to the method just outlined, showed a tendency for the strains to become later in maturity as they became more resistant. It therefore seemed evident that self-pollination and controlled cross-pollination of selected individuals offered possibilities for improvement which might not be attained by the other method. It should be possible to eliminate the undesirable recessive character of susceptibility by this means, while improvement in type by getting rid of certain undesirable characters might be expected. Should it be necessary to self-pollinate for many successive generations in order to reach the objective in mind, reduction in size and vigor of plants might occur. On the other hand, this obstacle might be overcome, if necessary, by carrying two or more distinct lines toward the same objective and then crossing two such lines when they had reached the desired degree of homozygosity, in order to restore vigor through heterosis.

METHODS OF EXPERIMENTATION

Cabbage under most conditions is a biennial. The simple and practicable method in the past was to make initial selections for resistance from a commercial field of the desired variety in which yellows had been very severe. These seed heads were placed in storage and set out the following spring and allowed to become cross-pollinated. Seed from each plant was saved separately and planted the following season on thoroughly sick soil in adjacent rows. The best plants from the most resistant rows were again saved and the process repeated until a highly resistant type was obtained. During the progress of the work now reported, certain revisions of this method have been adopted.

SEED PROPAGATION

The seed heads, instead of being held over winter in storage, are placed in the greenhouse for seed propagation during the winter months. This method offers three distinct advantages. It advances the rate of progress by allowing the progenies of one year's selection to be tested the following year. It precludes the necessity of prolonged winter storage of seed heads, which is especially difficult with the early, poorly keeping varieties. It facilitates greatly the practice of controlled pollination.

As the plants in the field approach maturity sufficiently to be judged for type, they are pulled and either planted in pots at once or more often held for some weeks in a cool cellar or in artificial cold

FIG. 1.—Cabbage seed plants in the greenhouse at Madison, Wis., April, 1926

storage. There is little advantage in potting the plants immediately, inasmuch as they require several weeks of dormancy before they go into flower and seed production. The potted plants are kept at comparatively low temperatures (10° to 15° C.) in order that the root system may become well established before the rapid growth of the top takes place. Plants selected during August and September are planted during October and are placed out of doors where they are allowed to remain until there is danger of injury from freezing. They are then removed to the greenhouse and kept at 10° to 15° C. until near blossoming time, when the temperature is raised to about 20°. By this means it is ordinarily possible to bring plants into blossom at Madison, Wis., in February. (Fig. 1.)

The manipulation of pollination is carried out in the usual manner. A portion of the seed plant, usually the central stem, is bagged and the pistils are artificially self-pollinated by working a camel's-hair brush over the open blossoms on alternate days. Such branches as are selected for crossing are bagged before blossoming begins, and

the buds are emasculated before the anthers become mature. Inasmuch as the indeterminate raceme grows rapidly at this stage, it is necessary to emasculate daily to avoid selfing. The pistils are receptive for several days, so that daily emasculation with pollination every second or third day ordinarily results in a satisfactory set of seed. As soon as the seeds have passed the "milk stage" the plants are cut and hung up in a warm, airy house to hasten maturity. By this means a majority of plants are ready to thresh by the last of May. Such seed may be sown at once, and plants are ready to set into the field during the last week of June or early July. Under Wisconsin conditions this program usually allows a thorough test for resistance and type during the current summer. Greenhouse propagation of cabbage seed has also been used with success by Melhus and his associates (*5*) at the Iowa Agricultural Experiment Station.

FIELD TESTS FOR RESISTANCE AND TYPE

In conducting the field tests, certain lines of procedure have developed during a period of several years which may well be described in some detail. The influence of soil temperature upon the incidence of cabbage yellows has been studied by Gilman (*1*), by Tisdale (*7*), and by Tims (*6*). In experiments where the soil was held at constant temperatures over a certain gradient it was found that the disease did not occur at 15° C., that it progressed slowly at 17°, and that it developed with greatest rapidity at 26° to 29°. The Wisconsin Hollander and Wisconsin All Seasons showed less resistance under these experimental conditions as the temperature was increased, and at the optimum for disease development there was nearly a complete breakdown in resistance. Of course, the entire root system is seldom if ever exposed to this extreme condition in nature. There were some differences in the severity of the disease at various soil moistures (*7*). In the soil which had a water-holding capacity of 31 per cent, the disease developed most rapidly and destructively at 15 per cent moisture, which was below the point where good normal growth of the plant was sustained; it was nearly as severe at 19 per cent moisture, where the best growth of the host occurred. At the latter soil-moisture content 100 per cent of the seedlings showed yellows, and 93 per cent had succumbed to the disease at the end of 28 days. In still higher moistures the disease was less destructive. At 23 per cent moisture an average of 89 per cent showed yellows and 64 per cent died, while at 26 per cent moisture 97 per cent were diseased and 68 per cent were killed. In the light of these facts, soil temperature and soil moisture may be expected to influence the development of the disease in the field as well as the expression of the resistant character.

Ordinarily under southeastern Wisconsin conditions the soil temperature is too low during late spring to permit development of yellows in the cabbage seed bed, while the most rapid development of the disease occurs usually in July during the three or four weeks following transplanting. On the other hand, in an occasional season the temperature during the middle or latter part of June is high enough to favor infection. In such an instance, were the seed bed made on infested soil, certain of the susceptible individuals would be eliminated before transplanting and more plants would succumb

in the moderately resistant and susceptible progenies than in the highly resistant ones. This would introduce an error into the comparison of those progenies in the field. The seed bed is therefore made on yellows-free soil and the plants transferred to infested soil for the resistance tests. Through a period of years it has been found that the most uniform and critical tests for resistance in the locality mentioned are obtained by sowing the seed from May 10 to May 30 and transplanting from June 25 to July 15.

The same plot of soil located in eastern Kenosha County, Wis., is used each year in making the field tests. By repeated cropping with cabbage this soil has become as thoroughly and uniformly infested with the yellows organism as is readily possible to attain under natural field conditions. For purposes of comparison a commercial (susceptible) variety and one of the resistant varieties now in general use, such as Wisconsin All Seasons, are planted at frequent intervals throughout the plot. The customary procedure in notation of yellows has been to determine the number of diseased plants in each progeny at intervals of 7 to 12 days after the first appearance of the disease. A gradual increase occurs in the number of diseased individuals until about the middle of August, the rate depending partly upon climatic conditions. During this period a gradation in the severity of the disease among individuals of a given progeny is usually noticeable. A number of the diseased plants are often killed within a few days after the appearance of the first symptoms, some persist for a few weeks, a few even produce small heads at the end of the season, while still others may show only slight symptoms on one or two lower leaves during the warmest part of the season. The last-mentioned type of plant eventually loses the affected leaves, and thereby any outward sign of the disease disappears, and with the return of cooler weather subsequent growth is normal. Whether this variation is a true gradation in resistance due to differences in hereditary factors, or whether certain environmental factors are responsible in whole or in part, is still an unsettled question. It is certain that soil temperature, soil moisture, and stage of growth of the host at the time of infection are important factors which influence the expression of resistance.

Variation from season to season and variation in time of planting within a single season have been found to influence the expression of resistance of a given progeny, and undoubtedly these differences are traceable to the varied effects of these environmental factors. It has therefore become a common practice, where the seed supply permits, to test the important progenies through two or three seasons. All plants which show even slight symptoms of yellows are labeled with small bamboo stakes as soon as the disease appears, so that if they recover later in the season they are avoided in making further selections. This custom of permanently marking all diseased plants was first adopted in 1924. (Fig. 2.)

Beginning with 1924, the plants affected by yellows were divided into four more or less arbitrarily established classes, namely, (1) those killed by the disease, (2) those so severely injured as to be unable to form heads, (3) those only slightly affected, which may form heads although they clearly show slight inhibitive effects of the organism, and (4) those in which the slight symptoms disappear during the course of the season and which head quite normally. This classification gives

a clearer picture of the damage caused by the disease in a particular strain than would be apparent in the mere record of the total percentage of plants affected. Thus, in a given strain showing 25 per cent of the plants affected, all or most of the cases might fall in the "recovery" class, whereas in another strain showing the same percentage of plants affected the majority might fall in the "dead" class. For purposes of comparison in this paper, the classification is simplified somewhat. In the field results of 1924 and subsequent

FIG. 2.—A portion of the Kenosha trial plot on yellows-sick soil. Note bamboo stakes used to mark each plant showing yellows. The two rows in the center contain highly resistant families, while at either side are very susceptible families

seasons, the plants falling in the "dead" and "severe" classes are combined in one category, and the "slight" and "recovery" classes are combined in a second group.

DESCRIPTION OF PARENT STOCKS USED

Cabbage varieties are commonly divided with respect to the length of their period of development into three groups—early, second early or mid season, and late. As previously noted, the need for yellows-resistant late varieties has been met for the present, and the work in progress has to be primarily with the mid-season and early sorts. In this report attention is given to the mid-season varieties. The market and sauerkraut demands within this group are not sufficiently uniform to justify concentrated attention upon selection within a single mid-season variety. In sauerkraut manufacture the preference is strongest for the flathead type, while in many cases the market demands are decidedly in favor of the roundhead varieties.

It seemed advisable, therefore, to make selections from varieties representing both types. For the flat type the All Head Early variety was chosen because of its popularity among sauerkraut packers and in certain cabbage-shipping sections. For the roundhead type Glory of Enkhuizen and Copenhagen Market were used. These last two are grown widely for mid-season shipping and for sauerkraut manufacture. Copenhagen Market properly belongs in the early group, but, as will be pointed out later, certain strains sold under this name really belong to the mid-season class. It is from one of these mid-season strains that the selections from Copenhagen Market herein described were made. A brief description of each of these parent varieties is given.

GLORY OF ENKHUIZEN

The variety known as Glory of Enkhuizen was introduced into America in the late nineties by Sluis & Groot, a seed firm of Enkhuizen, Netherlands. It produces a prominent spherical head which at maturity has a light-yellowish cast, and in most strains now in use there is sufficient "bloom" to give a bluish green tinge to the outer foliage. The stem is comparatively short, and at maturity the outer leaves are not very numerous, resulting in the characteristic prominence of the head. This variety has become very popular in recent years as a shipping cabbage following the early crop, such as early Copenhagen Market, Jersey Wakefield, and Charleston Wakefield, and preceding the late varieties such as Danish Ballhead. It is also used widely in sauerkraut manufacturing centers where it commonly shares in popularity with All Head Early and the mid-season strains of Copenhagen Market for the early pack of sauerkraut.

COPENHAGEN MARKET

Certain strains sold under the name of Copenhagen Market are close to Glory of Enkhuizen in type and maturity, and sometimes the two varieties are used interchangeably for market and for sauerkraut manufacture. Copenhagen Market was introduced into America in 1912 by Hjalmar Hartman & Co., of Copenhagen, Denmark. The original introduction was an early-maturing roundheaded cabbage with yellowish green foliage. Like the Glory of Enkhuizen, it produces a prominent head and comparatively few outer leaves at maturity. The original type, however, matures at least two weeks earlier than Glory of Enkhuizen and ordinarily produces a smaller head. Since the time of its introduction much deviation from the original type has appeared in many of the strains offered for sale. This has probably been brought about by laxity in selection, by mixture, and by substitution. In any case, at the present time two general types of Copenhagen Market are recognized. Certain of the strains now listed by seedsmen as "Extra Early Copenhagen," "Superselect Copenhagen," "Reselected Copenhagen," or with other qualifying terms conform closely to the original. Golden Acre, a recent introduction, seems to differ little from the original Copenhagen Market. Other strains produce larger heads, are more variable as a rule, and mature anywhere from a few days to two weeks later than the original strain; but they are still sold as Copenhagen Market. Such strains approach the characters of Glory of Enkhuizen and

really belong in the mid-season group. In this connection it may be well to cite the opinion of Work (*9*):

> Those who plant Copenhagen cabbage should take account of the fact that there are two distinct types of this variety. The one is early as Wakefield, is small, [and] matures very uniformly * * *. Golden Acre is practically the same as this strain, being, if anything, smaller and earlier. The second type is larger, later, and an excellent strain, but is sure to disappoint the man who works for early market. This is a type that is best suited for mid-season market and for the kraut factory.

In the present investigation with this variety original selections were made at Marion, Va., from stock labeled Copenhagen Market. It was evident from the beginning, however, that it was one of the later strains of the variety, and it was continued because of its intrinsic value as a mid-season type for shipping and local market as well as for sauerkraut. Thus the resistant strain Marion Market, though selected from Copenhagen Market, does not possess the early-maturity qualities of certain strains of the latter, and this fact should be clearly recognized in its use. Selection has been started from very early strains of Copenhagen Market, but these are being carried as distinct lines and will be reported upon later. The resistant strain Iacope, introduced recently by Melhus, Erwin, and Van Haltern (*5*), was also derived from Copenhagen Market.

ALL HEAD EARLY

The original selection of All Head Early was made in 1888 by W. Atlee Burpee, from a field of Henderson's Succession being grown in eastern Long Island by J. M. Lupton. It was introduced to the trade in 1891. It is one of the earliest of flathead types of cabbage and for that reason has become a popular variety for early sauerkraut. In the lots of this variety which have been studied considerable variability has been noted, and it does not appear to be a well-fixed variety. As a whole it may be considered as having a short stem, flat head with comparatively few outer leaves at maturity, petioles short, and foliage intermediate in color between the yellowish green of Copenhagen Market and the bluish green of Glory of Enkhuizen. Certain undesirable variations from this type will be discussed in connection with the detailed account of selections made from this variety.

DEVELOPMENT OF THE RESISTANT VARIETY GLOBE FROM GLORY OF ENKHUIZEN

FIRST-GENERATION SELECTIONS

Initial selections were made in 1921 from resistant individuals which remained healthy in a planting of very uniform commercial stock of Glory of Enkhuizen on the trial grounds in Kenosha County, Wis. This soil was thoroughly infested with the yellows fungus, and only a small percentage of the original plants survived. From these, several heads were selected which conformed reasonably well with the average type for the variety. They were stored over winter and planted the following spring in an isolated spot where cross-pollination was allowed to take place. Head-to-row tests were made in 1923 from the six plants producing seed. The results, recorded in Table 1, show that all progenies had considerable resistance.

Retrials in 1924 and 1925 indicate that the last two seasons offered conditions for a more severe test than did 1923. These first selections all conformed reasonably well to the standard type. Two progenies, however (35–1 and 2) showed a tendency of the heads to flatten slightly, and they were therefore discarded. From the four remaining lots about 100 heads were saved for greenhouse seed propagation in the winter of 1923–24.

TABLE 1.—*Behavior of first, second, and third generation progenies of Globe selected from Glory of Enkhuizen variety of cabbage on yellows-infested soil*

Generation	Parent strain	Progeny number [a]	1923 trials		1924 trials			1925 trials			1926 trials		
			Total number of plants	Yellows (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)
First	Commercial Glory of Enkhuizen.	35–1	246	16									
		35–2	254	18	219	15	5	65	23	6			
		35–3	255	[b] 20	40	28	3	49	22	6			
		35–4	254	[b] 14									
		35–5	257	[b] 21	46	15	0	26	31	0			
		35–6	513	[b] 15									
Second	35–3 to 6	35–11s			24	13	0	26	4	8			
		35–11×12			105	1	0	75	1	1	46	0	0
		35–12s			69	0	[b] 0	52	0	[b] 2			
		35–12×11			25	0	0	219	0	[b] 0			
		35–12×13						51	0	0			
		CG–7[c]×35–11			62	18	0						
		CG–7×35–12			50	0	0						
		CG–7×35–13			67	42	15						
Third	35–12×11	35–26–B									18	22	0
	35–12s	35–22s									17	0	0
		35–22×21						25	0	0			
		35–43s									8	0	0
		35–43×59									13	0	0
		35–51s									20	25	0
		35–58s									21	0	0
		35–59s									19	0	0
		35–67s									17	0	0
Commercial.			251	73	48	60	19	214	53	30	240	61	27
Wisconsin All Seasons.			258	1	55	0	2	50	0	4	23	0	0

[a] The suffix s indicates that the progeny is the result of self-pollination of a given plant. When a progeny is the result of a cross between two plants, e. g., 11×12, the number of the pistillate parent is given first. Progenies 35–1 to 35–6 are from plants 1 to 6 which were allowed to cross-pollinate.

[b] Further selections were made at the end of the season.

[c] CG–7 was a plant from the commercial variety Glory of Enkhuizen, the selfed progeny of which showed practically 100 per cent yellows on sick soil, indicating that it was homozygous for susceptibility.

SECOND-GENERATION SELECTIONS

The mortality[5] of seed plants in the greenhouse was very heavy, and only two of the second-generation selections produced seed (35–11 and 12), while a third (35–13) produced enough pollen to use in certain crosses. In addition to selfing each plant and crossing within the group, crosses were made with a plant of a susceptible

[5] Decay of the stem in the region of and below the head is common in the greenhouse. It occurs after the mother seed heads are planted and either during the dormant period or after elongation of the seed stem has started. No single organism is responsible for this injury. Bacteria and fungi, including *Phoma lingam* and Rhizoctonia spp., are commonly associated with the decay.

strain. Tests of these progenies were made in 1924 and 1925, and the results are included in Table 1.

The progeny from the selfing of plant 35–11 showed some susceptibility to yellows, while progeny 35–12s was highly resistant.

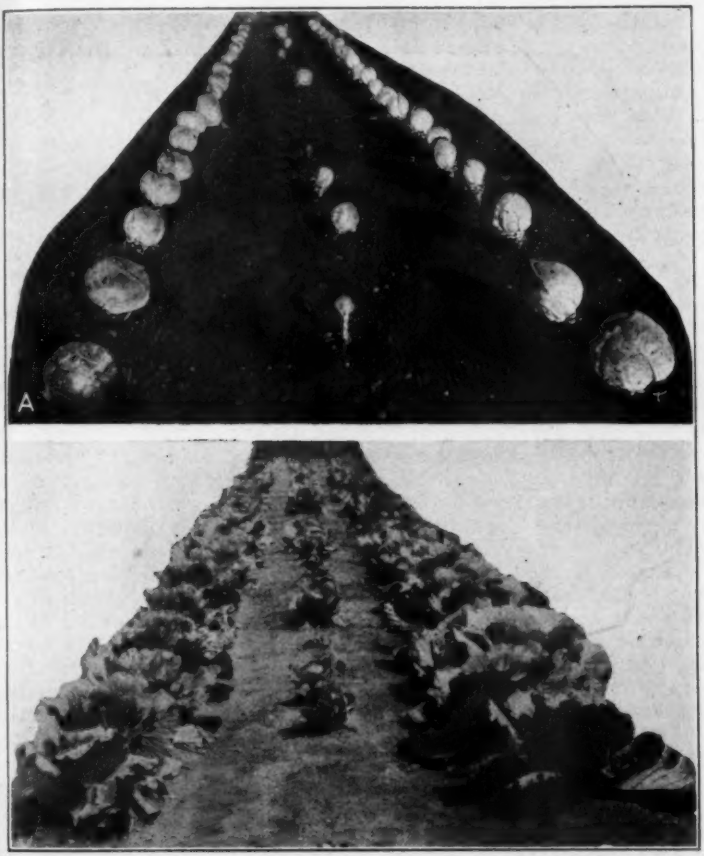

FIG. 3.—Progeny tests of midseason cabbage strains on Kenosha trial plot in 1925. Bamboo markers were removed before the photographs were taken: A, Center row is commercial Copenhagen Market, which showed 96 per cent of plants yellowed. At the right is Globe progeny 35-12×11 (see Table 1), selected from Glory of Enkhuizen completely free from disease. At the left are hybrids from a cross between 35–12 which was homozygous for resistance and a Copenhagen Market plant which was heterozygous for resistance. Note that resistance is completely dominant. B, Center row is commercial Golden Acre, of which 83 per cent of the plants were diseased. At the left is Marion Market progeny 30–13×15B (selected from Copenhagen Market), which showed one slight case of yellows out of 47 plants. At the right are All Head Select progenies, which were highly resistant

Hybrid plants from crosses in which plant 35–12 entered into the combination also proved to be highly resistant. In Figure 3, A, progeny 35–12×11 is shown in comparison with commercial Copenhagen Market and with the hybrids from a cross between a partially resistant plant and plant 35–12. Other experiments to be reported

later have shown that whenever a plant which is homozygous for resistance is crossed with a heterozygous one or one homozygous for susceptibility the F_1 hybrid plants are resistant. It is to be noted here that when plant 35–12 was crossed with a susceptible plant the F_1 hybrid plants were all resistant. This indicates that plant 35–12 was homozygous for resistance. The F_1 hybrid from crosses between plants 11 and 13 and the susceptible parent showed marked degrees of susceptibility, indicating that they were still heterozygous for resistance. The selfed progeny from plant 12 (35–12s) was very uniform and satisfactory in type, and this was therefore used as a basis for further selections. Plants from this lot were saved in 1924 and 1925, and several third-generation progenies were thus obtained. In addition a number of plants from progeny 35–12 × 11 were saved and later cross-pollinated, the seed being grouped into one lot, 35–26–B.

THIRD-GENERATION SELECTIONS

Inasmuch as progeny 35–12 × 11 was the result of a cross between a homozygous and a heterozygous parent, a certain amount of reversion would be expected in the next generation. This proved to be the case when lot 35–26–B was tested and found to have 22 per cent yellows in the "dead or severe" class. (Table 1.) The progenies derived from 35–12s all proved to be 100 per cent resistant with the exception of 35–51s, which again segregated at the ratio of 3 resistant to 1 susceptible. This should not have been the case if plant 35–12 were truly homozygous. An explanation of this variation is offered by the possibility that it was the result of contamination in the pollination of plant 35–12. Such chance contamination is not entirely eliminated and was particularly likely to occur in 1924–25 greenhouse propagation, when all the selfed branches were not bagged during the entire blossoming period. The supposition is supported in this case by the facts that the segregation was exactly in the 3 to 1 ratio and this particular progeny showed marked deviation in type from 35–12s while the sister progenies continued to resemble the mother progeny quite uniformly. It is likely that with the exception of 35–51s the third-generation progenies are homozygous for resistance under field conditions.

TYPE OF THIRD-GENERATION PROGENIES

The third-generation progenies show a marked degree of uniformity in type. Typical heads are shown in Figure 4, A, B. The color is very close to that of the average Glory of Enkhuizen, while time of maturity and shape and texture of head all seem to be reasonably similar in the two strains. The prominence of the head at the time of maturity is not as pronounced in the selected progenies as is often noted in standard Glory of Enkhuizen. This is due largely to the fact that the new strain has a few more leaves in the outer foliage, which does not constitute a serious objection. With the exception of progeny 35–51s, all third-generation progenies resemble very closely the characters of 35–12s and 35–12 × 11. It is evident that pure-line selection for two successive generations has, in addition to fixing resistance, served to fix certain characters which go to make up type. By this means much of the variability in cabbage varieties

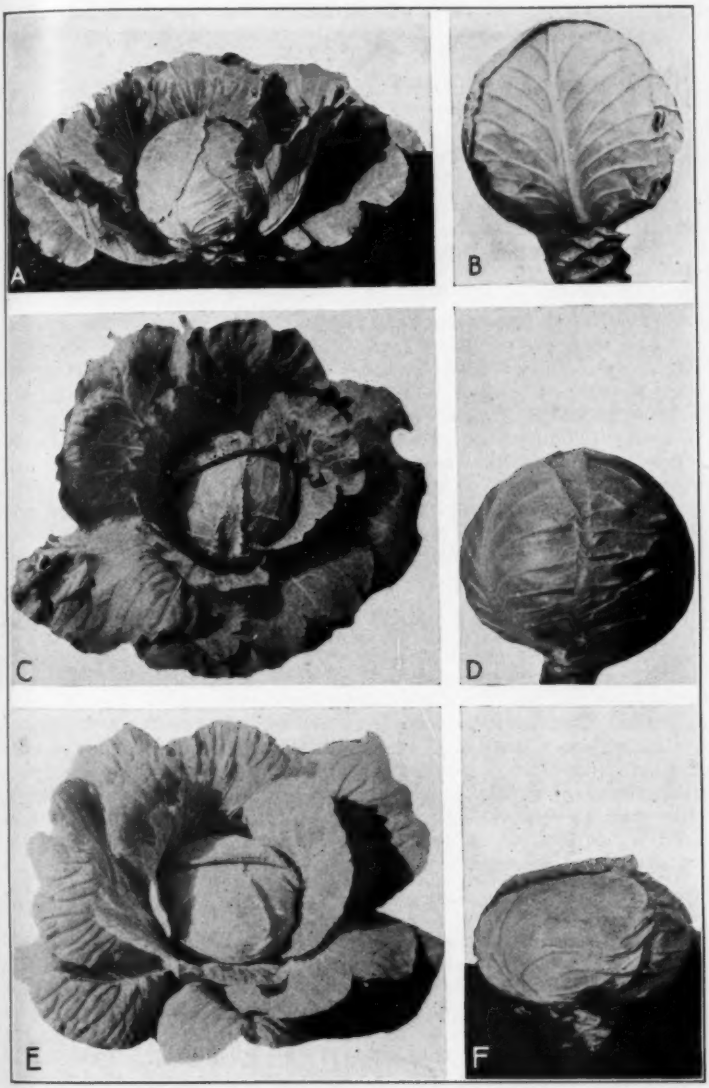

FIG. 4.—Typical plants from the yellows-resistant midseason varieties: A, B, Globe, a roundhead type selected from Glory of Enkhuizen; C, D, Marion Market, a roundhead type selected from Copenhagen Market; E, F, All Head Select, a flathead type selected from All Head Early. Compare with Figure 5

may undoubtedly be eliminated. There is little evidence that the third-generation progenies are seriously reduced in vigor as a result of selfing for two successive generations.

MULTIPLICATION OF GLOBE

It is evident that the selections within this variety (to which the name Globe has been given) have reached a point where little may be expected from further pure lining. The present plan, therefore, is to multiply the third-generation progenies (except 35–51s). The plants from these progenies will be allowed to cross-pollinate in order to provide a greater quantity of seed and to insure against reduction in vigor. The seed thus procured may serve as stock seed for multiplication under proper safeguards in one or another cabbage-seed growing region.

Because of the urgent demand for resistant strains of this type a number of heads from strains 35–3 to 6 were selected for type in 1923, and seed was produced from them in 1924. A trial of this stock seed in the Kenosha plots in 1925 showed only 12 per cent of the plants severely diseased, while 80 per cent remained healthy, and the type, though not equal to the best progenies tested in 1926, was quite as satisfactory as commercial Glory of Enkhuizen. The remainder of this stock was sent to the Puget Sound region in the spring of 1925 by the National Kraut Packers' Association, and a crop of seed was obtained in 1926. Thus this advance lot of seed will be available at once for preliminary use on a commercial scale until the multiplied seed from the third-generation progenies becomes available.

DEVELOPMENT OF THE RESISTANT VARIETY MARION MARKET FROM COPENHAGEN MARKET

Initial selections were made in 1921 from a commercial planting of Copenhagen Market in a demonstration plot conducted near Marion, Va., under the direction of F. D. Fromme, of the Virginia Agricultural Experiment Station. The original stock was one of the later maturing strains of Copenhagen Market, having on an average a bluish green rather than a yellowish green foliage, although variation in this respect was evident from the beginning. Selection has now passed through three generations. The results of the trials on the Kenosha grounds are given in Table 2.

FIRST-GENERATION SELECTIONS

The original heads selected at Marion in 1921 were held over winter in storage and planted together in an isolated spot in 1922, where they were allowed to become cross-pollinated. Head-to-row progeny tests were made in 1923 from the six plants maturing seed (30–1, 2, 3, 4, 5, 6). All six progenies showed high resistance, the percentage of yellows varying from 11 to 18. Retrials of certain of the progenies in 1924 and 1925 showed higher percentages of disease, indicating that yellows was not generally as severe in 1923 as in the two following seasons. In the 1923 trials these progenies showed a fairly satisfactory approximation to the type of the late strains of Copenhagen Market. They were decidedly later than the early strains of the variety and in fact were scarcely earlier than the

first-generation progenies of the Globe selections from Glory of Enkhuizen tested on the same trial grounds in the same year. There was some variation in color, but in the main the plants were more bluish green than those of the standard Copenhagen Market. Two of the progenies (30–22–4 and 5) averaged somewhat later in maturity than the rest and showed some tendency to produce flattened rather than spherical heads and were therefore discarded. From the remaining four progenies about 100 of the best plants were saved for greenhouse seed production during the winter of 1923–24.

TABLE 2.—*Behavior of first, second, and third generation progenies of Marion Market selection from Copenhagen Market variety of cabbage on yellows-infested soil*

Generation	Parent strain	Progeny number [a]	1923 trials		1924 trials			1925 trials			1926 trials		
			Total number of plants	Yellows (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)
First	Commercial Copenhagen Market.	30–1	250	[b] 14	22	36	5						
		30–2	249	[b] 16	68	19	0	97	25	0			
		30–3	253	[b] 10									
		30–4	248	[b] 18									
		30–5	254	14									
		30–6	133	14									
Second	30–1, 2, 3, 4	30–12s			25	0	[b] 0						
		30–12×14B			62	0	0	50	0	0			
		30–13s						50	0	[b] 0			
		30–13×15B						47	0	4			
		30–14s			34	12	[b] 0	50	22	0			
		30–14×12B			29	17	0	212	25	3			
		30–14×17B						79	29	3			
		30–15s						50	18	8			
		30–15×13B						50	24	4			
		30–16s						50	0	0			
		30–16×18B						25	0	0			
		30–17s						75	24	0			
		30–17×14B						34	24	0			
Third	30–12s	30–25–A									84	0	0
	30–14s	30–30s						26	0	0			
		30–31s						53	4	2			
		30–32s						50	18	2			
		30–33s						22	32	6			
		30–35s						25	24	4			
	30–13s	30–36s									51	0	0
		30–37s									49	0	0
		30–38s									114	25	0
		30–38×37									36	0	0
		30–40s									29	0	0
		30–41s									133	26	2
		30–40×41B									139	0	0
		30–41×40B									150	25	2
		30–53s									48	0	0
Commercial.			251	72	48	60	19	214	53	30	240	61	27
Wisconsin, all seasons			258	1	55	0	2	50	0	4	23	0	0

[a] The suffix s indicates that the progeny is the result of self-pollination of a given plant. When a progeny is the result of a cross between two plants, e. g., 38×37, the number of the pistillate parent is given first. In some cases the blossom of the pistillate parent was not emasculated, but a brush was worked back and forth between blossoms of paired plants, and such progenies are therefore a mixture of plants resulting from selfing of the pistillate parent and from crossing with another staminate parent. Such a progeny is designated by the suffix B, e. g., 13×15B. Progenies 30–1 to 30–6 are from plants 1 to 6 which were allowed to cross-pollinate.

[b] Further selections were made at the end of the season.

SECOND-GENERATION SELECTIONS

The mortality in the greenhouse planting was very heavy, and only six plants produced seed. Each of the plants produced some seed from self-pollinated blossoms. Certain crosses were made by working a single brush over groups of blossoms on paired plants. Such combinations are designated as 30–12 × 14B, etc., the number of the pistillate parent being given first in each case and the suffix B being added to distinguish them from crosses in which the flowers of the pistillate plants were emasculated before pollination. The seed produced in such crosses was therefore partly from self-pollination and partly from cross-pollination.

Four of the progenies were tested in 1924, and all but 30–12s were tested in 1925. Three of the selfed lots showed complete resistance, while the other three were clearly heterozygous for resistance. There was also marked segregation in type characters. Progeny 30–12s was planted so late in 1924 that the plants did not mature heads and therefore could not be judged critically for type. There was not sufficient seed for a second trial in 1925, but several plants from the 1924 plantings were saved and grown in an isolated spot in 1925, where they were allowed to become cross-pollinated. Progeny 30–13s, in addition to being completely resistant, was quite uniform in other characters. The color was distinct from that of the majority of the other lots in being yellowish green, approaching that of the standard early Copenhagen Market. The time of maturity appeared to be slightly earlier than the average of the sister progenies and apparently a few days earlier than the Globe line, though it was distinctly later than the earliest strains of Copenhagen Market. The plants were larger and somewhat leafier than the last-named variety, but the heads were typical. (Fig. 4, C, D.) This appeared to be by far the best progeny when resistance and type were both considered. Several third-generation selections were made from it. A trial row of 30–13 × 15B alongside nonresistant Golden Acre is shown in Figure 3, B. Lot 30–14s showed extreme deviation. The color of the foliage was distinctly more bluish than is the rule, while the heads assumed a distinctly oblong shape. (Fig. 5, D, E, F.) The 1924 trial was planted too late to judge for type, but several plants were saved and seed produced from five in the winter of 1924–25. Progeny 30–15s approached 30–13s, but was not highly resistant and not as uniform in type. Lots 30–16s and 30–17s approached 30–14s as to type characters.

THIRD-GENERATION SELECTIONS

Of the three progenies selected for continuation, 30–13s gave most promise both for type and resistance. Progenies 30–12s and 30–14s were planted too late in 1924 to judge for type, but retrial of 30–14s in 1925 showed it to possess certain undesirable qualities. Strain 30–25–A, resulting from 30–12s plants, proved to be highly resistant (Table 2), but since the heads were decidedly flattened it was not carried farther. The progenies from 30–14s (30–30 to 35) showed that in all but one there was, as in the parent progeny, a distinct tendency to produce oblong heads, while segregation was also apparent in color of foliage and in resistance. They were not continued.

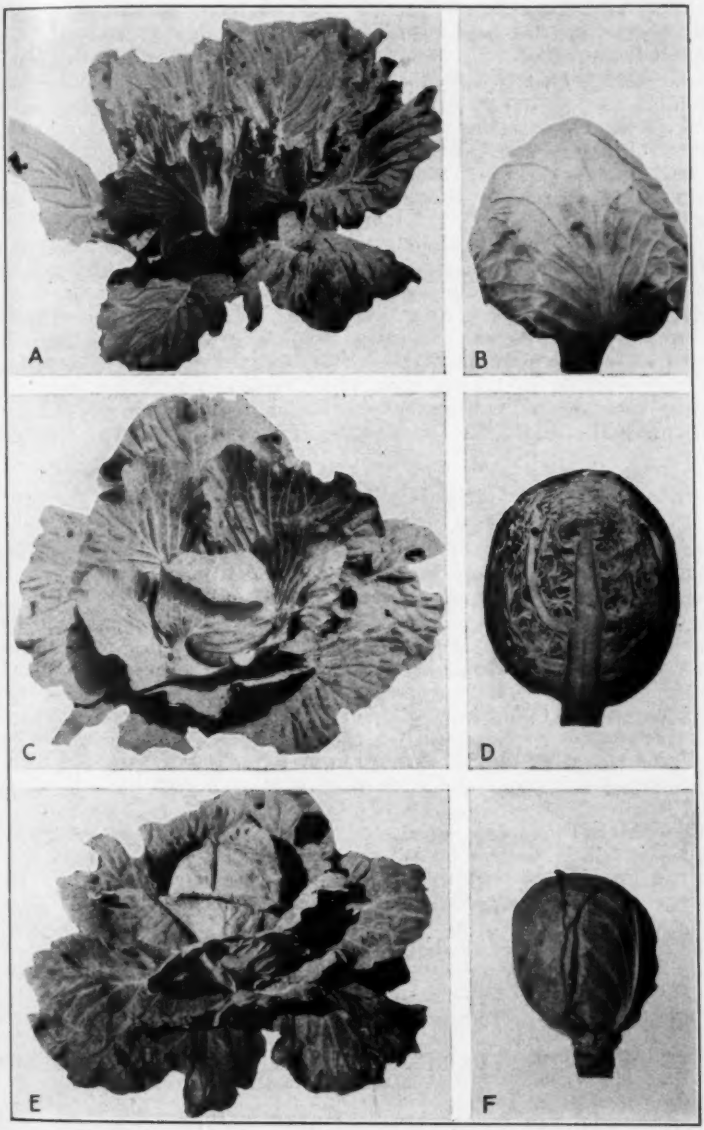

FIG. 5.—Examples of atypical plants which predominated in certain progenies and necessitated disposal of the latter in spite of high resistance. A, All Head Early plant showing long petioles. (Compare with fig. 4, E.) B, pointed head which predominated in a homozygous resistant line selected from All Head Early. (Compare with fig. 4, F.) C, misshapen head common to many progenies from All Head Early. (Compare with fig. 4, E.) D, E, F, three views of elongated head type prevalent in several progenies selected from Copenhagen Market. Note the excessively long core in a head of this type. (Compare with the more desirable type preserved in Marion Market, fig. 4, C, D)

The third-generation progenies derived from 30–13s all showed characters of the parent strains in remarkable uniformity. The yellowish green foliage was prevalent, and the nearly spherical head which flattened very slightly on top with maturity was predominant. Two of the six progenies (30–38s and 30–41s) segregated at the ratio of 3 resistant to 1 susceptible. It is possible that this was the result of pollen contamination of parent plant 30–13, the selfed branches of which were not bagged during the entire period of blossoming. Lots 30–36s and 30–37s were preserved for further seed production.

It is evident from this analysis that the first-generation selections were much improved in degree of resistance. Possibly the crossing of the plants through open-pollination tended to obscure the heterozygous condition as to other characters. The production of selfed progenies in the second generation resulted in a marked segregation both as to resistance and type. By selfing for one more generations it was possible to approach or attain homozygosity for resistance along with greater uniformity of other characters than prevailed in the original stocks. This was particularly true of progenies derived from 30–13s.

TYPE AND MULTIPLICATION OF MARION MARKET

Further multiplication of this variety (Marion Market) is to be based upon progenies 30–36s and 30–37s. Plants from these two lots will be used to produce stock seed which may serve as a basis for increase crops. In the meantime preliminary trials on a commercial scale are made possible by seed procured in 1924 from surplus heads selected from progenies 30–1 to 4. Part of this was planted in the trial plots in 1925. Though not as uniform in type as 30–36s or 30–37s, it was quite as good as the parent variety and showed 82 per cent healthy plants and only 14 per cent severely diseased. The remainder of the seed was placed in the Puget Sound seed-growing region in Washington State in 1925 by the National Kraut Packers' Association, and a crop of seed was obtained therefrom in 1926.

The Marion Market variety as represented by progenies 30–36s and 30–37s is a mid-season variety maturing at the same time as or only a few days earlier than the Globe. (See fig. 4, C, D.) The foliage is yellowish green with comparatively little bloom and is very similar in these respects to most strains of Copenhagen Market. The head is spherical or slightly flattened on the top. The stem is medium in length, and the core extends to about the center of the head. The leaves of the head, as in Globe, are unevenly crumpled and not smooth and tightly overlapping.

DEVELOPMENT OF THE RESISTANT VARIETY ALL HEAD SELECT FROM ALL HEAD EARLY

FIRST AND SECOND GENERATION SELECTIONS

Initial selections were made in 1920 from the surviving plants of All Head Early planted in the trial plot of infested soil. The seed plants were allowed to cross-pollinate in 1921, and in 1922 head-to-row tests were made from five individuals (40–21–1 to 5). None of these showed any marked improvement in resistance over the com-

mercial variety. (Table 3.) One lot (40–21–5) was made the basis of further selection because it contained the largest percentage of plants of desirable type. These second-generation heads were grown for seed in the greenhouse, where the plants were selfed and certain crosses made.

TABLE 3.—*Behavior of first and second generation selections from All Head Early variety of cabbage on yellows-infested soil*

First-generation selections, trial in 1922			Second-generation selections, trial in 1923		
Progeny No.[a]	Total number of plants	Yellows (per cent)	Progeny No.[b]	Total number of plants	Yellows (per cent)
			40–1s	85	70
			40–1×6	65	0
			40–1×7	51	15
40–21–1	157	75	40–4s	20	[c] 10
40–21–2	231	69	40–4×6	18	[c] 0
40–21–3	245	66	40–4×7	31	[c] 18
40–21–4	263	64	40–6×7	14	[c] 0
40–21–5	265	[c] 71	40–7s	53	[c] 20
			40–7×3	25	15
			40–7×6	39	[c] 0
Commercial All Head	105	77		213	90
Wisconsin All Seasons	364	8		258	1

[a] The parent plants of these progenies were allowed to cross-pollinate.
[b] See Table 2 for explanation of progeny numbers.
[c] Further selections continued.

The second-generation progenies (Table 3) showed much improvement in resistance; 40–4s and 40–7s appeared to segregate roughly at the ratio of 3 resistant to 1 susceptible, indicating that the plants were heterozygous for resistance. Wherever plant 40–6 entered into crosses the progeny was completely resistant, indicating that it was homozygous for resistance. Unfortunately, no selfed progency from this plant was obtained. Segregation for type characters was also evident. Lots 40–4s and 40–4 × 6 were of the most satisfactory type. Progeny 40–7s and crosses in which 40–7 was one of the parents were not true as to color and showed a tendency to produce misshapen heads. Further selection from several progenies was continued, as indicated in Table 3.

THIRD-GENERATION SELECTIONS

The results of trials of the third-generation progenies on infested soil in 1924 and 1925 are given in Table 4. A perusal of these data shows that several progenies were obtained which appeared to be highly resistant to yellows. Thus lots 40–93s, 40–120s, 40–130s, and crosses in which 40–130 served as one parent, 40–137s, 40–199s, and most of the crosses in which 40–80 entered as a parent, showed very few diseased plants. Most of them, however, showed one or more undesirable qualities, such as off color, too long petioles, misshapen heads, or late maturity. Some of these undesirable characters are illustrated in Figure 5. Certain other progenies, not so highly resistant, were much superior in type, of which the most promising were 40–76s, 40–91s, 40–91 × 93, and 40–96s.

TABLE 4.—*Behavior of third-generation selections from All Head Early variety of cabbage on yellows-infested soil*

Parent strain	Progeny No.[a]	1924 trials			1925 trials		
		Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)
40-4s	40-75s	29	17	27			
	40-76s	8	0	[b] 12			
	40-80×76	9	0	0			
	40-80×79	15	0	0			
	40-80×81	29	0	0			
	40-80×82	51	0	2	23	0	0
	40-82×80	41	2	12	47	0	0
40-4×6	40-90s	14	14	29			
	40-91s	45	7	[b] 9			
	40-91×93	74	4	[b] 11	50	0	[b] 6
	40-93s	19	21	0			
40-4×7	40-96s	24	13	[b] 17			
	40-97s	19	0	11			
40-6×7	40-112s	16	6	25			
	40-114s	14	0	7			
	40-115s	13	0	38			
	40-120s	11	0	0			
40-7s	40-131s	62	19	55	51	14	17
	40-137s	82	0	[b] 0	50	0	2
	40-130s	31	3	0			
40-7s and 40-4s	40-130×75	61	0	0			
	40-130×79	28	0	0			
	40-130×82	36	0	0			
40-7×6	40-199s	36	0	11	49	0	0
	40-207s	18	19	19			
	40-212s	32	0	19			
Commercial All Head Early		23	95	0	112	54	46
Wisconsin All Seasons		55	0	2	50	0	4

[a] See Table 2 for explanation of progeny numbers. [b] Further selections continued.

Inasmuch as the aim in this improvement work was to procure strains acceptable as to type as well as resistance, it was not considered advisable to continue those progenies which showed distinctly undesirable qualities even though they were highly resistant. All of these except 40–137s are therefore dropped from further consideration in this discussion, even though certain of them were continued for other purposes. Progeny 40–137s, though slightly off in certain respects, was continued. The major emphasis was turned to further selection from the somewhat less resistant lots 40–76s, 40–91s, 40–91×93, and 40–96s. From these, fourth-generation trials were made in 1925 and 1926.

FOURTH AND FIFTH GENERATION SELECTIONS

Most of the fourth-generation selections were grown to seed in the greenhouse in the winter of 1924–25 and the first trials made in 1925. Some additional selections from 40–91×93 were made in the summer of 1925, and the new progenies thus obtained were not tested until 1926. A summary of the results of these trials is given in Table 5. It will be seen that the lots derived from 40–137s continued to fall in the highly resistant class. They were not improved

in type, however, over the parent progeny. Of those derived from the less resistant lots (40–76s, 40–91s, 40–91×93, and 40–96s), some were no more resistant than the parent stocks, while others showed little or no disease. In general, the better qualities of the parent strains were preserved. In 1925 further selections were made from 40–303×353, and 40–326s. The trials of the resulting progenies are given in the same table. While they were not all free from disease, all but 40–512s and 40–538s showed only slight traces of yellows.

At the end of the 1926 season a number of families were on hand which were highly resistant to yellows and at the same time conformed very uniformly to the desired type. The best strains were those which were derived originally from 40–4×6 (Table 3) and then from 40–91×93. (Table 4.) Certain of these were of the fourth generation and others of the fifth generation. As they had been subjected to inbreeding for these several generations, the expected reduction in vigor was evident. Further pure-line selection might bring further reduction and thus offset the gain acquired by improved uniformity in type and resistance. At the end of the 1926 season, therefore, four progenies were selected for their combination of good type with resistance. There were two fourth-generation selections (40–406s and 40–406×407) and two fifth-generation selections (40–508×512 and 40–511×510). A portion of each of these is being grown in mixed plantation in 1927 to produce stock seed for multiplication.

It is hoped that this combination, even of closely related progenies, will result in restoring any loss of vigor which became apparent in the fourth and fifth generations as a result of selfing. As a safeguard against the possibility that such a combination of strains will not sufficiently restore loss of vigor, a portion of the heads will be planted with heads selected from 40–535s. The latter is derived by a line of selection distinct from that of the others. It is highly resistant and not greatly removed from the other strain in type.

TYPE AND MULTIPLICATION OF ALL HEAD SELECT

As already pointed out, the four best strains from the 1926 trials are to be combined to produce stock seed in 1927. This seed will serve as a basis for multiplication. In the meantime the urgent demands for seed of a resistant strain of this type justified steps to multiply some of the less completely finished progenies. Accordingly, about 100 heads were selected from the trials of 1923 (Table 3), and from them a quantity of stock seed was grown in 1924. When tested in 1925 it proved to be highly resistant and fairly uniform in type. Most of this lot was placed for seed growing in the Puget Sound region in 1925 by the National Kraut Packers' Association, and a crop of seed was obtained in 1926. Similarly, the best heads not taken for special purposes from the 1924 trials (Table 4) were grown for stock seed in 1925 and a second multiplication crop started in 1926. These advance strains of the variety All Head Select will be available for general use until the improved selections of 1926 can be multiplied.

The type of All Head Select as represented in strains 40–406s, 406×407, 508×512, and 511×510 is very similar to that of 40–91×93 shown in Figure 4, E, F. The strain is very similar to the original All Head Early. The stem is short, the head fairly flat, and

the color of foliage intermediate between the yellowish green of Copenhagen Market and the bluish green of Glory of Enkhuizen. The outer leaves at maturity are comparatively few and the petioles very short, giving a compact plant with a fairly prominent head. It matures about the same time as the parent variety and close to Globe and Marion Market. It is two weeks or more ahead of Wisconsin All Seasons or Wisconsin Brunswick and thus supplements the last two varieties in season.

TABLE 5.—*Behavior of fourth and fifth generation selections from All Head Early variety of cabbage on yellows-infested soil*

Generation	Parent strain	Progeny No.[a]	1925 trials			1926 trials					
						First planting			Second planting		
			Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)	Total number of plants	Yellows, dead or severe (per cent)	Yellows, slight or recovered (per cent)
Fourth	40-76s	40-302s	24	0	25	....	....	....	....	....	....
		40-302×306	24	8	4	....	....	....	....	....	....
		40-302×307	26	0	20	....	....	....	....	....	....
		40-303s	16	0	[b]0	....	....	....	....	....	....
		40-303×353	15	0	[b]7	....	....	....	....	....	....
		40-303×304	12	0	0	....	....	....	....	....	....
		40-303×310	25	0	0	....	....	....	....	....	....
		40-304×310	25	12	0	....	....	....	....	....	....
		40-305s	28	7	11	....	....	....	....	....	....
	40-91s	40-308s	50	0	4	....	....	....	....	....	....
		40-309s	25	0	4	....	....	....	....	....	....
		40-308×309	27	4	11	....	....	....	....	....	....
		40-310s	25	0	8	....	....	....	....	....	....
	40-91×93	40-325s	15	0	20	36	6	3	....	....	....
		40-325×326B							25	0	0
		40-326s	15	0	[b]0	18	0	6	....	....	....
		40-326×329B				40	0	3	....	....	....
		40-329s	21	0	10	....	....	....	....	....	....
		40-329×326B	25	0	16	....	....	....	....	....	....
		40-402s	....	....	....	20	0	0	....	....	....
		40-403s	....	....	....	7	0	0	....	....	....
		40-405s	....	....	....	33	0	6	26	4	0
		40-406s	....	....	....	17	0	0	24	0	0
		40-406×407	....	....	....	64	0	9	25	0	0
		40-407s	....	....	....	34	0	0	....	....	....
		40-408s	....	....	....	20	0	20	....	....	....
		40-410s	....	....	....	19	0	0	25	0	16
		40-410×411	....	....	....	32	0	6	....	....	....
		40-411s	....	....	....	....	....	....	25	0	0
	40-96s	40-334s	30	0	0	....	....	....	....	....	....
		40-335s	27	0	0	....	....	....	....	....	....
	40-137s	40-353s	24	0	4	....	....	....	....	....	....
		40-354s	33	0	3	....	....	....	....	....	....
		40-355s	34	3	0	....	....	....	....	....	....
		40-421s	....	....	....	....	....	....	23	0	0
		40-426s	....	....	....	....	....	....	25	0	0
Fifth	40-326s	40-508×512	....	....	....	16	0	0	54	0	7
		40-511s	....	....	....	9	0	11	....	....	....
		40-511×510	....	....	....	48	0	0	61	0	0
		40-512s	....	....	....	15	7	7	....	....	....
	40-303×353	40-535s	....	....	....	....	....	....	23	0	0
		40-538s	....	....	....	....	....	....	21	5	0
Commercial			215	51	34	51	61	14	240	61	27
Wisconsin All Seasons			100	0	6	74	0	5	23	0	0

[a] See Table 2 for explanation of progeny numbers. [b] Further selections continued.

DISCUSSION

The results of several seasons' experimentation support the earlier supposition that there exists in most if not all varieties of cabbage some degree of natural resistance to the Fusarium disease, and that by selection of individuals which survive upon infested soil, resistant strains may be readily developed. Results with the development of the three new mid-season varieties emphasize the necessity of pure-line selection, and it is only by such methods that one may hope to fix resistance in a given strain. It appears that resistance as expressed under field conditions in southern Wisconsin is governed by a single dominant factor, and thus homozygous lines may be expected with two or three generations' selections.

The danger of digression in type during the progress of selection for resistance is equally evident. There is little to support the theory that resistance is linked with important type characters. On the other hand, most commercial cabbage varieties are more or less heterozygous for various characters, and segregation is commonly found when pure-line selections for resistance is started. This may appear disadvantageous, but it likewise may be beneficial, inasmuch as it offers the opportunity to reject undesirable qualities which might otherwise be masked because of their recessive nature. The results show that if one so desires he may by this means improve the uniformity in type of a given strain at the same time that he is selecting for resistance. It is certain that selection for resistance alone may defeat its own end by so changing the type through indiscriminate perpetuation of undesirable individuals as to reduce the commercial value of the resulting strain. The danger of reduction in vigor from repeated selfing imposes a limitation to indefinite perpetuation of selfed lines. It may be advantageous in some cases, as has been suggested with All Head Select, to carry parallel but distinct selfed lines which may be intercrossed eventually when each has reached a desirable degree of uniformity.

The three mid-season varieties All Head Select, Marion Market, and Globe have been advanced to a high degree of resistance and of uniformity of type. They appear to be nearly if not completely homozygous for resistance under the average field environment in which they were selected. It has been shown by Tisdale (7) that the resistance of Wisconsin Hollander may be broken down when plants are grown at high constant soil temperatures under greenhouse conditions, and similar results were attained when seed was sown in a naturally infested field during midsummer. In certain commercial cabbage sections the plants are probably exposed to a higher temperature at an earlier stage in their development than in southern Wisconsin. The period of exposure to favorable conditions for the disease may therefore be longer and the extreme temperatures may be higher. Just how much these varied environments will inhibit the commercial value of these new varieties remains to be determined through repeated testing by those who may be interested in their use. Variation in the causal organism, especially with respect to its selective pathogenicity, must also be looked upon as a possible factor influencing their behavior in different localities.

Confidence in the usefulness of these new varieties in latitudes south of Wisconsin is based upon field trials carried on in cooperation

with pathologists of other States. These have included critical tests adjacent to susceptible commercial strains on yellows-infested soil in northern and central Indiana, northern Ohio, Maryland, southwestern Virginia, western Tennessee, southeastern Iowa, and northeastern Kansas. In all cases these trials followed the period and methods used for the commercial crop in each locality. With the exception of the Muscatine, Iowa, trials of 1926, where the incidence of yellows was somewhat higher than in the Kenosha plot,[6] the three varieties stood up as well in other localities as they did in southeastern Wisconsin. It is believed, therefore, that they will usually prove satisfactory for commercial use on infested soil.

SUMMARY

The main purpose of the present paper is to report the essential details which concern the development of three new Fusarium-resistant varieties of cabbage, all of which are classified in the mid-season group.

The methods of selection, hybridization, and field testing are described.

The Globe variety is a selection from Glory of Enkhuizen, a commercial variety of the round-head type. After three generations' selection, progenies were obtained which resemble the parent variety in its main features and were perfectly free from signs of yellows in the 1926 trials.

In like manner the Marion Market was brought to the same degree of resistance from original selections out of Copenhagen Market, a commercial variety of the round-head type. The type, though distinct from some of the early-maturing strains of the parent variety, is well suited for use as a mid-season type.

All Head Select was selected from the flathead commercial variety All Head Early. This strain has given the greatest difficulty in securing the combination of desirable type and resistance. Certain fourth and fifth generation progenies are satisfactory and are to be multiplied.

In all three varieties the trials of 1926 offered the most advanced and satisfactory selections. These will be multiplied for commercial use. In the meantime certain less-advanced progenies of each variety have been multiplied by the National Kraut Packers' Association and are now available for general use.

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BACTERIAL STRIPE BLIGHT OF OATS[1]

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INTRODUCTION

In the issue of Phytopathology for September, 1918,[3] a "stripe blight" of oats was mentioned by the writer as occurring in Wisconsin and adjoining States. The signs of the disease were described as distinctly different from those of halo blight of oats [4] but the causal organism was not named or described. Since that time specimens of this leaf disease have been sent in from various parts of the United States, and the causal organism has been studied in its relation to *Bacterium coronafaciens* Elliott and to the ability of the two organisms to infect various varieties of oats and other Gramineae.

SIGNS OF THE DISEASE

The lesions first appear as sunken, water-soaked dots, which, when very numerous, cause the collapse of the leaf tissue, or, when coalescing, form long water-soaked stripes (fig. 1) or blotches which may show narrow yellowish margins. Under moist conditions, especially early in the morning, bacterial exudate stands out in drops along the lesions. This exudate dries down to thin white scales, and the lesions as they become older turn a rusty translucent brown. The streaks vary in length from a fraction of an inch to several inches or may extend the length of the leaf blade and down to the sheath. Halolike borders do not develop about these lesions.

Lesions may occur on plants in all stages of development from seedlings to mature plants. While the lesions are found chiefly on the leaves, occasionally they occur on sheaths, culms, pedicels, and glumes. Sometimes the entire top of a plant is killed by the water-soaked lesions which spread over the culm and the sheath, inclosing the panicle. The long, water-soaked, stripelike lesions, the absence of a halo, and the presence of exudate serve to distinguish stripe blight from halo blight of oats. (Fig. 2.)

GEOGRAPHICAL DISTRIBUTION

Stripe-blight lesions were first observed and infected leaves were collected by A. G. Johnson at Urbana, Ill., and Wooster, Ohio, during the season of 1917. From that year to the spring of 1926,

[1] Received for publication June 16, 1927; issued December, 1927.

[2] The writer is indebted to A. G. Johnson, of the Office of Cereal Crops and Diseases, for collecting diseased plants and for helpful suggestions.

[3] ELLIOTT, C. BACTERIAL OAT BLIGHT. Phytopathology 8: 489-490. 1918.

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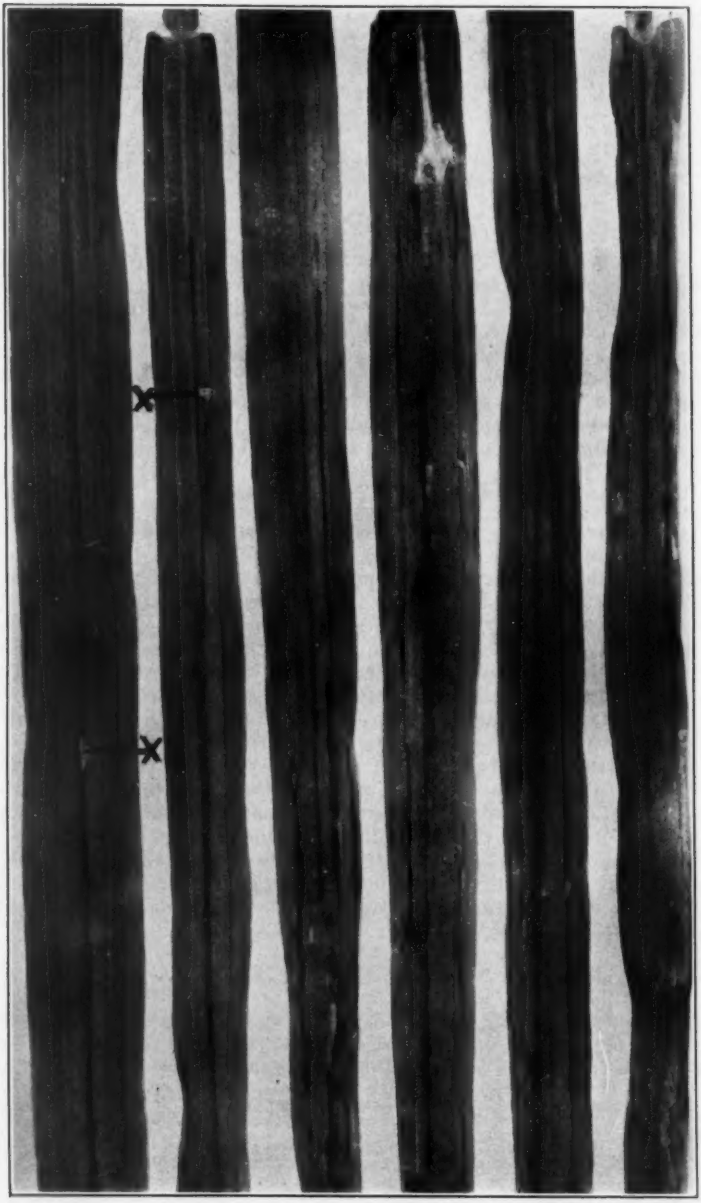

FIG. 1.—Stripe-blight lesions and exudate (X) from natural infections on leaves of Wisconsin No. 146 oat (*Avena byzantina* C. Koch) collected at Madison, Wis., June 13, 1918. × 2

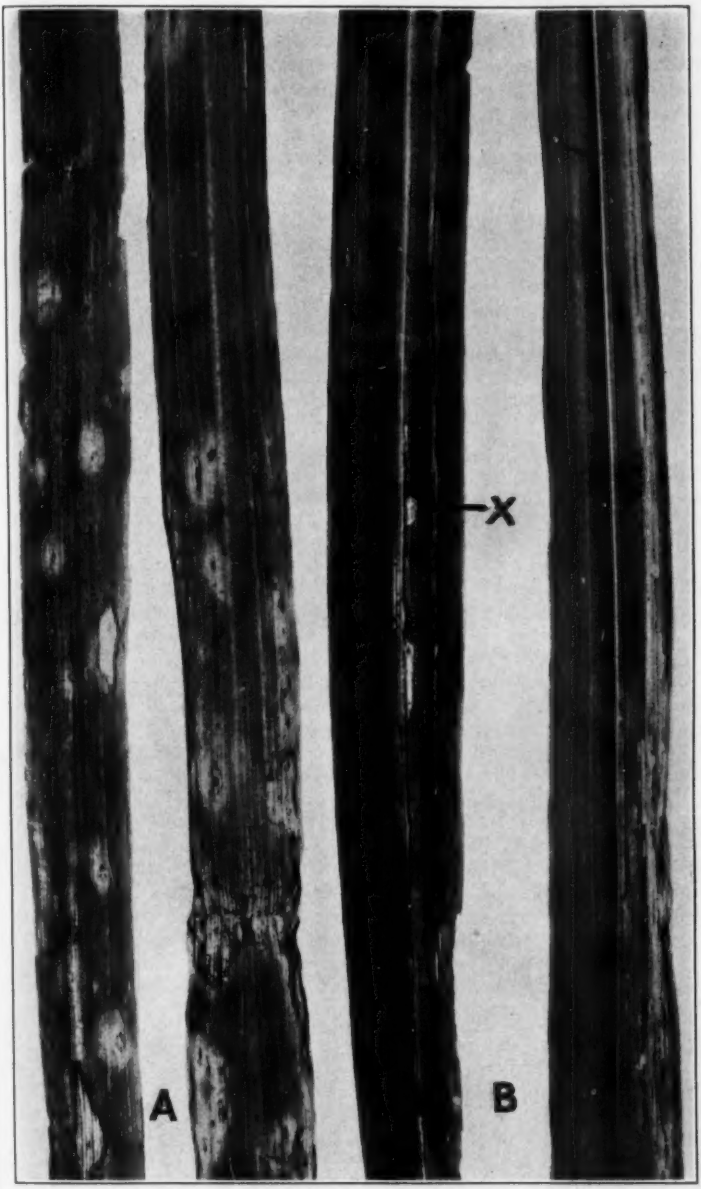

FIG. 2.—Halo-blight lesions (A) and stripe-blight lesions (B) from artificial inoculations with *Bacterium coronafaciens* (isolation No. 74) and with *Bact. striafaciens* (isolation No. 10), respectively, on leaves of Wisconsin Pedigree No. 14 oat (*Avena sativa*). Photographed October 11, 1924. Natural size

oat leaves containing lesions of stripe blight have been collected in different parts of the country, in almost every year, and on plants from the seedling stage to those nearing maturity. Table 1 shows the localities from which specimens have been received and the varieties infected.

TABLE 1.—*Collections of stripe blight of oats, showing oat varieties, dates and localities of collection, and names of collectors*

Variety	Date	Locality	Collector
Swedish Select	June 20, 1917	Urbana, Ill.	A. G. Johnson.
Not known	June 21, 1917	La Fayette, Ind.	Do.
Wideawake	June 23, 1917	Wooster, Ohio	Do.
Not known	July 14, 1917	Custer, S. Dak.	C. W. Hungerford.
Wisconsin No. 146 (Avena byzantina).	June 10, 1918	Hill farm, Madison, Wis.	Charlotte Elliott.
Not known	June 15, 1918	College Hill, Madison, Wis.	Do.
Wisconsin No. 146 (Avena byzantina).	do	do	Do.
Not known	June 25, 1918	Wooster, Ohio	A. D. Selby.
Wisconsin Pedigree No. 14	July 17, 1918	Madison, Wis.	Charlotte Elliott.
Not known	Mar. 29, 1919	Davis, Calif.	A. G. Johnson.
Do	Apr. 2, 1919	do	Do.
Red Rustproof (Avena byzantina).	June 7, 1920	Granite City, Ill.	A. G. Johnson, H. B. Humphrey, and H. H. McKinney.
Not known	do	Madison, Wis.	Charlotte Elliott.
Wisconsin No. 103	June 9, 1920	do	Do.
Not known	June 28, 1920	Hill farm, Madison, Wis.	Do.
Sixty-Day	May 26, 1921	Mandan, N. Dak.	A. G. Johnson.
Big Four and Silvermine	June 18, 1924	La Fayette, Ind.	A. G. Johnson and E. B. Mains.
Silvermine	June 21, 1924	De Kalb, Ill.	C. S. Reddy and A. G. Johnson.
Not known	do	Chenoa, Ill.	A. G. Johnson.
Do	do	Bloomington, Ill.	C. S. Reddy and A. G. Johnson.
Do	June 22, 1924	Oregon, Wis.	A. G. Johnson.
Do	June 23, 1924	Madison, Wis.	Do.
C. I. 620 (unnamed)	June 26, 1924	University farm, St. Paul, Minn.	J. J. Christensen and A. G. Johnson.
Black Tartarian	do	St. Paul, Minn.	A. G. Johnson and O. S. Aamodt.
Guyra	Apr. 30, 1925	Davis, Calif.	A. G. Johnson.
White Ligowa	May 1, 1925	do	Do.
Red Rustproof [Texas Red, Calif. No. 1003 (Avena byzantina)].	do	do	Do.
Garton Yellow, C. I. No. 1612.	May 9, 1925	Corvallis, Oreg.	Do.
Belyak, C. I. No. 1899	do	do	Do.
Markton, C. I. No. 2053	do	do	Do.
Black Mesdag, C. I. No. 1877	May 12, 1925	Pullman, Wash.	E. F. Gaines and A. G. Johnson.
Not known	Apr. 20, 1926	Davis, Calif.	W. W. Mackie.

THE CAUSAL ORGANISM

ISOLATIONS

From stripe-blight lesions, collected on several oat varieties from different parts of the country, more than 40 isolations have been made. The organism is not sensitive to sterilization. The leaf tissue may be dipped in 95 per cent alcohol, put into mercuric chloride solution (1 : 1,000) for two minutes, washed, and then crushed in broth, from which the plates are poured. While yellow colonies were observed on some of the plates, the characteristic white colonies were much more numerous and often appeared in pure culture. In many cases plates were poured from halo and stripe lesions at the same time. The white colonies developing from both types of

lesions were always so nearly alike that they could not be distinguished. Colonies begin to appear on the plates after about 48 hours. They are raised, white, smooth, shining, and round or with slightly undulate thin margins. (Fig. 3, D.) The narrow radiate border shown in Figure 3, C, is characteristic, especially of isolation No. 66b. There also are more or less well-developed internal fish-scale markings similar to those of halo-blight colonies.

INOCULATIONS

Inoculations were made by spraying oat plants with water suspensions of the organisms from young agar slants. The plants were then kept in damp chambers for about 48 hours. Controls were sprayed with sterile water and kept under similar conditions. Halo-blight organisms were used for comparison in the same inoculation experiments with the stripe-blight organisms, and they always produced abundant typical halo lesions. Infections with both organisms began to appear in from three to five days. The lesions of stripe blight first appeared as water-soaked dots, 1 millimeter in diameter, which turned brown with translucent centers and sometimes had narrow yellow margins. These centers of infection increase until they are 1 to 2.5 centimeters in length, or they sometimes may extend one-half to three-fourths the length of the leaf. The long narrow lesions are brown and water-soaked, with narrow yellow-brown margins. Where lesions are very numerous, the whole leaf turns yellow, with points of infection evident, and then collapses. Small dried scales of exudate are apparent at points of infection.

Thirty-five inoculations with the stripe organism were made in the greenhouse and 16 in the field. Of the 35 greenhouse inoculations 5 produced no lesions, owing to unfavorable conditions of temperature and moisture or to resistant varieties of oats. Of the 30 successful inoculations, 8 produced only scattered but typical lesions and the remainder moderate to heavy infection.

The lesions produced by these inoculations always were typical of stripe-blight lesions. (Fig. 2, B.) No halo-blight lesions ever developed from inoculations with the stripe-blight organism, although typical halo-blight lesions were produced on the same varieties inoculated at the same time with the halo-blight organism. (Fig. 2, A.) In the field it is more difficult to control environmental conditions, and so the results of inoculations are less satisfactory. Of the 16 field inoculations, 6 were negative and 10 positive, only 2 of the latter showing heavy infection.

Table 2 shows the results of one inoculation experiment which was made both in the greenhouse and in the field in the spring of 1926. Ninety-two varieties and strains, including most of the cultivated species and varieties of oats, were sprayed with water suspensions of both halo and stripe organisms, to learn whether there would be any varietal differences in susceptibility to the two organisms. In all varieties the susceptibility to the two organisms was nearly or quite the same, so that the organisms could not be separated on this basis. Where there were some differences in host reaction in the greenhouse, a second set of inoculations was tried, but these only confirmed the previous conclusion that each variety reacts in about the same way to both organisms.

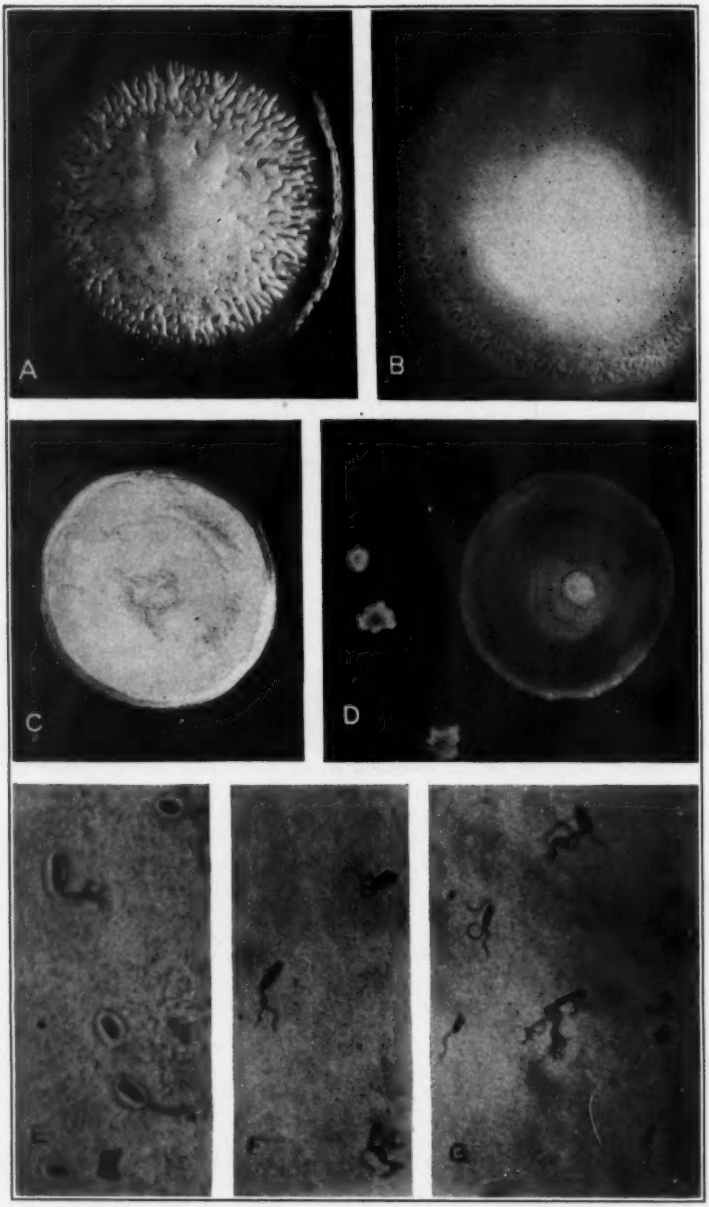

FIG. 3.—A, *Bacterium striafaciens*, 6-day colony of isolation No. 10 on Thaxter's potato agar. Photographed by direct light Oct. 17, 1924. × 5. B, *Bact. coronafaciens*, 6-day colony on Thaxter's potato agar. Photographed by direct light Oct. 17, 1924. × 5. C, *Bact. striafaciens*, 3-day colony of isolation No. 66b on +15 beef-peptone agar. Photographed by transmitted light. × 5. D, *Bact. striafaciens*, 18-day colony of isolation No. 66b on +15 beef-peptone agar. Photographed by transmitted light. × 6. E, *Bact. striafaciens* from 3-day culture of isolation No. 14, grown on beef-peptone agar and stained by Casares-Gil carbol-fuchsin method, showing capsules. × 1,700. F and G, *Bact. striafaciens* from 24-hour culture of isolation No. 66b grown on beef-peptone agar and stained by Casares-Gil carbol-fuchsin method, showing flagella. × 1,550

TABLE 2.—*Degrees of infection of 92 varieties and strains of oats grown in the greenhouse and in the field at Arlington Experiment Farm, Rosslyn, Va., and inoculated with the organisms causing halo blight and stripe blight, respectively, to determine any differences in susceptibility to the two organisms*

[Degrees of infection: 0=None, 1=slight, 2=moderate, 3=heavy, 4=very heavy]

Species and variety	C. I. No.	Degree of infection					
		In greenhouse				In field June 8	
		Feb. 5		Feb. 19			
		Halo blight	Stripe blight	Halo blight	Stripe blight	Halo blight	Stripe blight
Avena byzantina:							
Coastblack	1025	0	0	2	0		
Black Algerian	840-1	1	2			1	1
Sterilis Selection (of Etheridge)	1859-1	0	1			1	1
Red Rustproof	1039	1	2			0	1
Red Rustproof (of Etheridge)	1860	1	0			0	0
Fulghum	708	0	0	0	0	0	0
Burt (of Etheridge)	1861	1	1			0	1
Sunrise (Calif. No. 1012)	1799	1	2			1	1
Culred	518-189	1	1			1	1
Avena sativa:							
Boswell	480	2	2			0	0
Black Winter	606	0	3	1	2	1	1
Hatchett	838	0	1			1	1
Tech	905	2	2			1	1
Winter Turf	1570	2	0			0	0
Custis	2041	2	1			1	1
Lee	2042	2	1			1	1
Culberson	273	0	0	1	0	0	0
Dwarf Culberson	748	0	0	1	0	1	1
Bicknell	206-151	1	1			2	2
Black Mogul	1074	2	4			1	1
Black Bell	1767	2	3			0	1
Victor	803	2	3			0	1
Black Mesdag	1877	2	3			1	1
Monarch	1876	1	1			1	1
Joanette (of Etheridge)	1880	1	2			1	1
Early Joanette	1092	1	2			1	1
Colburt	2019	0	0	0	0	1	0
Black Diamond	1878	0	2			1	1
Monarch Selection (of Etheridge)	1879	1	2			2	2
Old Island Black	1756	2	2			1	1
Do	2035	2	1				
North Finnish	1882	3	2			1	1
Ruakura	2025	1	0			1	1
Cornellian	1242	1	0			0	0
White Maine	1692	3	3			1	1
Richland	787	3	3			2	2
Kherson	1148	1	2			2	2
State Pride	1154	3	3			1	0
Navarro (Ferguson Navarro)	966	0	0	0	0	0	0
Markton	2053	3	3			2	2
Madrid	603	3	3			1	1
Aurora	831	0	0	0	0	0	0
Golden Drop	1890	3	2			1	1
Green Russian	1978	3	1			0	0
Iogren	2024	1	1			1	1
Japan	1157	4	1			0	1
Minota	1285	1	0			1	1
Awnless Probsteier	1888	2	1			1	0
Probsteier	16°6	3	2			2	1
June (of Etheridge)	1902	3	1			1	1
Wernich Golden	1746	2	1			1	1
Garton No. 473	1613	2	2			1	1
Albion	729	3	2			1	1
Cole	834	3	3			1	0
Sixty-Day	1887	1	1			1	0
Iowar	847	3	1			1	0
Early Ohio	1117	3	3			2	1
Early Champion	1623	3	2			1	1
Daubeney	1621	1	1			1	1
Yakutsk	498	1	1			1	0
White Bonanza	1686	1	0	0	0	1	1
Canadian	1625	1	1			0	0
Castleton Potato	1972	2	1			1	1

TABLE 2.—*Degrees of infection of 92 varieties and strains of oats grown in the greenhouse and in the field at Arlington Experiment Farm, Rosslyn, Va., and inoculated with the organisms causing halo blight and stripe blight, respectively, to determine any differences in susceptibility to the two organisms*—Continued

[Degrees of infection: 0=None, 1=slight, 2=moderate, 3=heavy, 4=very heavy]

Species and variety	C. I. No.	Degree of infection					
		In greenhouse				In field June 8	
		Feb. 5		Feb. 19			
		Halo blight	Stripe blight	Halo blight	Stripe blight	Halo blight	Stripe blight
Avena sativa—Continued.							
Early Mountain	1624	0	1			1	1
Tobolsk	1709	1	1			0	0
Wisconsin Wonder	1645	2	1			0	0
Silvermine Selection (of Etheridge)	1894					0	0
White Avalanche	1440	0	0			1	1
Irish Victor	1896	2	1			1	1
O. A. C. No. 72	846	1	1			1	1
Scottish Chief	1699	1	1			1	1
Gothland	1898	3	3			2	1
Minnesota No. 295	1290	1	2			0	0
Danish Island	1684	1	1			1	1
Pringle Progress	1717	2	1			1	1
Belyak	1630	2	1			2	1
Swedish Select	1375	0	0	1	1	0	0
Colorado No. 37	1640	1	0			0	1
Lincoln	1262	1	0			1	0
Silvermine	1013	2	0			1	0
Comewell	1317	1	1			1	1
Victory	1145	0	0	2	2	1	0
Tabor	1777	1	2			1	0
Avena sativa orientalis:							
Storm King	1602	2	2			1	1
Tartar King	1599	2	1			1	1
Marvelous	1999	0	2			1	2
Green Mountain	1872	0	3	3	1	1	1
White Tartar	1614	1	1			0	1
Avena nuda:							
Hoejer (Hull-less)	1003	0	0	0	0		0
Kilby (Hull-less)	1995	1	0				0
Liberty (Hull-less)	845	1	2				
Fowlds (Hull-less)	1996	0	1				
Hull-less	1770	0	1				

Inoculations were made on *Bromus inermis* Leyss, *B. carinatus* Hook and Arn., *Agropyron repens* (L.) Beauv., and *Chaetochloa lutescens* (Weigel) Stuntz (*Setaria glauca* of authors). No lesions were produced on any of these hosts, although inoculations on oats at the same time produced definite stripe-blight and halo-blight infections.

Inoculations with the stripe-blight organism (66b) were made on tobacco, cauliflower, cabbage, and broomcorn, but with negative results.

Inoculations with the stripe-blight organism (isolation No. 14) and the halo-blight organism (No. 75) were made on *Avena strigosa* Schreb. and Markton oats and also on unidentified varieties of wheat, rye, and barley in the greenhouse. Barley and oats showed slight infection with the stripe-blight organism and oats and rye with *Bacterium coronafaciens*.

COMPARISON WITH OTHER ORGANISMS

H. R. Rosen in 1919[5] and 1922[6] described a bacterial disease of foxtail (*Chaetochloa lutescens*), the causal organism of which he said was infectious to many Gramineae, including oats, on which the lesions were not unlike those produced by *Bacterium coronafaciens*. The spots on oats, he says, may vary from indefinite light-yellow areas to withered grayish green with marked tinges of red when the infected areas coalesce. Ordinarily there is no exudate. Infections by *Bact. coronafaciens* on oats show no exudate, but are definite oval yellow halos about sunken tissue at the points of infection. The lesions produced on oats by the foxtail organism are evidently quite different from the water-soaked dots and translucent brown streaks of the stripe organism. The occurrence of abundant exudate under favorable conditions also is a distinguishing feature.

The stripe-blight organism differs from *Pseudomonas alboprecipitans* in important morphological and cultural characters, as recorded by Rosen. The writer was not able to obtain a culture of the foxtail organism for comparative cultural and inoculation tests.

According to Reddy and Godkin,[7] the brome-grass organism, *Bacterium coronafaciens* var. *atropurpureum*, differs from *Bact. coronafaciens* only in host reactions. The latter oat organism infected only one species of brome grass (*Bromus carinatus*), while the brome-grass organism infects both brome grass and oats. The kinds of lesions produced on oats by the two organisms are not identical. Those produced by the brome-grass organism have a more water-soaked appearance and narrower halos than those produced by the oat organism. On *B. inermis* the lesions are at first circular to elliptical water-soaked areas with yellowish green halos. Later these spots become dark chocolate or purplish brown and usually are linear. Similar lesions occur on *Agropyron repens* (L.) Beauv., on which they are dark purplish brown to black. From this description of the lesions it is evident that those caused by the brome-grass organism are distinctly different from the water-soaked dots and streaks produced by the oat-stripe organism.

CULTURAL AND MORPHOLOGICAL CHARACTERS

Through all the tests on culture media the stripe-blight organism and *Bacterium coronafaciens* were carried along together, but only slight differences were observed.

In dimensions, the stripe-blight organism was consistently smaller, having average measurements of 0.66 by 1.76 μ. *Bacterium coronafaciens* grown at the same time and under the same conditions and stained in the same way had average measurements of 0.68 by 2.40 μ. The organisms were grown for one or two days on beef-peptone and potato agar and were stained with gentian violet.

Both organisms have capsules (fig. 3, E) and one to several polar flagella (fig. 3, F). They are Gram-negative and not acid fast. Growth on beef-peptone agar is white and slightly raised, with entire to slightly undulate margins and internal fish-scale markings. Agar

[5] ROSEN, H. R. A PRELIMINARY NOTE ON A BACTERIAL DISEASE OF FOXTAIL. Science 49: 291. 1919.
[6] ROSEN, H. R. A BACTERIAL DISEASE OF FOXTAIL (CHAETOCHLOA LUTESCENS). Ann. (n. s.) Bot. Gard., Missouri 9: 333-402, illus. 1922.
[7] REDDY, C. S., and GODKIN, J. A BACTERIAL DISEASE OF BROME-GRASS. Phytopathology 13: 75-86, illus. 1923.

usually is somewhat greened, but only slightly as compared with the bright green produced by *Bacterium coronafaciens* var. *atropurpureum*. On Thaxter's potato agar, *Bact. coronafaciens* showed less wrinkling of the surface than the stripe-blight organism. (Fig. 3, A, B.) In beef-peptone bouillon, tubes at first become clouded at the top and bottom with a space between which is only slightly clouded. In three-day cultures, clouding at the bottom of the tube is moderate and in layers and extends about one-third of the distance to the top. Growth in the upper part of the medium is mostly at the surface in the form of a ring or thin pellicle which falls when disturbed. Clouding becomes more uniform as the age of the cultures increases.

In gelatin stab cultures of the stripe-blight organism, growth is best at the top. Liquefaction begins in 2 days and is completed in about 18 days at 20°–22° C. Liquefaction is crateriform, later becoming more infundibuliform to saccate. Cultures of *Bacterium coronafaciens* are only three-fourths liquefied in about 18 days.

PHYSIOLOGY

The optimum temperature for growth of both organisms is about 22° C.; the maximum temperature, between 33° and 35° C. Both grew at 1° C.

The thermal death point is about 48° C. Throughout the tests the stripe organism grew after exposure to slightly higher temperatures than *Bacterium coronafaciens*, but only about 1 degree higher.

The optimum reaction for both organisms is between P_H 6.5 and P_H 7.0. Both grew at P_H 5.5 but not at P_H 5.0, and both grew at P_H 8.9 but not at P_H 9.3.

Both organisms grown in Dunham's solution and tested by Ehrlich's method[8] gave negative reactions for indol. A culture of *Bacillus coli*, grown and tested at the same time, gave a positive reaction.

Seven strains, three of halo blight and four of stripe blight, were grown on lead-acetate agar with a reaction of P_H 7.0. All showed a distinct browning of the surface growth and a decided browning of the upper part of the agar as compared with the controls. A small quantity H_2S was produced by both organisms.

Both organisms are aerobes. Growth occurs only at the surface in stab cultures and in shake cultures of beef-peptone agar plus 1 per cent of glucose, lactose, and mannit, respectively.

In milk cultures both organisms occasionally clear without formation of curd. Usually, however, a soft curd forms in three to five days, followed by slow peptonization, which is completed in from one to two months.

Litmus milk begins to turn blue in two to three days. Reduction begins in about one week and is completed in three to seven days. The blue color gradually returns, first as a light grayish blue, which later becomes dark grayish blue.

Reduction of methylene blue begins in 24 to 48 hours and is completed in about 24 hours. The color returns slowly as a greenish blue. Several tests of cultures of both halo-blight and stripe-blight organisms grown in nitrate bouillon were made with starch water,

[8] Ehrlich's test.—One cubic centimeter of a 2 per cent solution of paradimethyl aminobenzaldehyde in 95 per cent alcohol is added to the culture. Then drop by drop, not more than 0.5 c. c. of concentrated hydrochloric acid is added until a red zone appears between the alcohol and peptone solution.

potassium iodide, and sulphuric acid. The results were negative with all oat cultures, while cultures of *Bacillus coli* gave positive reaction for nitrites. Cultures then were tested on the first, second, fourth, eighth, and tenth days with sulphanilic acid and *a*-naphthylamine. Tests on the eighth and tenth days both gave positive results, showing that with this test both the stripe-blight organism and *Bacterium coronafaciens* should be recorded as nitrate reducers.

Streaks of both organisms on starch agar plates tested with iodine after 12 days showed a clear zone for a centimeter from the margin of the colony.

Several tests were made to detect if possible any difference in acid or alkali production of the two organisms.

Tests for production of ammonia were made in the following ways:

Flasks containing seven-day broth cultures were heated and the steam passed into tubes of Nessler's solution. A yellowish brown precipitate was formed from both cultures and uninoculated controls, but there was much less precipitate from the controls.

Slant cultures then were made on the following medium: Peptone, 40 grams; glucose, 2 grams; dipotassium phosphate, 5 grams; agar, 15 grams; water, 1,000 cubic centimeters.

Tested with 1 per cent phenol and sodium hypochlorite, respectively, the cultures gave negative results, but with Nessler's solution a deep-orange precipitate formed in the inoculated tubes and none in the controls.

The following tests then were made to determine any differences in quantities of ammonia produced by the halo-blight and the stripe-blight organisms.

Cultures were grown in flasks containing beef-peptone broth, asparagin + dextrose in tap water, asparagin + dextrose in distilled water, 1 per cent asparagin in tap water, oat juice diluted, and oatmeal broth. Cultures of different ages were tested for the number of milligrams of nitrogen per liter by distilling off the ammonia into N/10 sodium hydroxide, methyl orange being used as indicator. No significant differences were found.

Tests for the production of acid were made with saccharose, dextrose, levulose, maltose, lactose, glycerin, and mannit in 2 per cent solutions plus 1 and 2 per cents of Difco peptone and Witte's peptone. In all tests there was a strong acid production from saccharose and dextrose and less acid from levulose. The reaction from the other compounds was always alkaline.

Tests then were made to determine whether there were differences in the quantities of acid produced by the two organisms from saccharose and dextrose. In one test with 2 per cent saccharose and 2 per cent dextrose plus 0.2 per cent Difco peptone in distilled water, brom cresol purple and brom phenol blue were added as indicators. In the dextrose cultures containing brom cresol purple, two of the five strains showed a higher acidity than the others, but one was a halo-blight organism and the other a stripe-blight organism. With saccharose the two halo-blight organisms were slightly more acid than the stripe-blight organisms. The acid range of brom phenol blue is too high to show the acid production from saccharose of the

oat organisms, but from dextrose the acid production was about the same for both organisms.

Tests with maltose and lactose in 1 per cent peptone solutions showed alkaline reactions from both organisms.

TABLE 3.—*Acid production, from different sugars in peptone solutions, by stripe-blight and halo-blight organisms, determined by the colorimetric method*

Date inoculated and medium used	Incubation period (days)	P_H concentration of medium												
		After inoculation with stripe-blight organism isolation No.—							After inoculation with halo-blight organism isolation No.—					Uninoculated
		66b	69	70	9	10	11	12	Stock	57a	74	75	76	
June 6, 1923:														
Saccharose (2 per cent) peptone (1 per cent)	1	6.2	6.0	6.1	----	----	----	----	6.2	----	----	----	----	6.0
	2	5.8	5.8	6.1	----	----	----	----	6.3	6.2	----	----	----	6.1
	3	5.8	5.7	6.0	----	----	----	----	6.2	6.2	----	----	----	----
	5	5.5	5.8	5.9	----	----	----	----	6.0	6.0	----	----	----	6.1
	9	5.4	5.4	5.7	----	----	----	----	5.8	5.2	----	----	----	6.0
	26	5.2	----	5.3	----	----	----	----	5.2	5.3	----	----	----	----
Nov. 6, 1924:														
Dextrose (2 per cent), Difco peptone (0.2 per cent)	2	----	----	----	6.0	6.0	5.7	----	----	----	5.2	5.6	----	5.8
	4	----	----	----	6.2	6.2	5.1	----	----	----	4.8	5.5	----	5.8
	7	----	----	----	6.2	6.0	4.4	----	----	----	4.2	5.3	----	----
	9	----	----	----	6.1	6.0	4.4	----	----	----	4.2	5.4	----	5.6
	11	----	----	----	5.7	6.0	4.4	----	----	----	4.1	5.0	----	5.7
	14	----	----	----	5.3	5.8	4.1	----	----	----	4.1	5.0	----	----
	18	----	----	----	5.1	5.6	4.1	----	----	----	4.1	4.5	----	5.7
	22	----	----	----	5.1	5.4	4.1	----	----	----	4.0	4.4	----	5.6
Nov. 6, 1924:														
Dextrose (2 per cent), Difco peptone (0.2 per cent), tartrate (0.5 per cent)	2	----	----	----	6.1	6.2	6.2	----	----	----	5.7	6.1	----	6.1
	4	----	----	----	6.1	6.3	5.7	----	----	----	5.7	6.1	----	6.1
	7	----	----	----	5.9	6.1	5.6	----	----	----	5.6	5.9	----	----
	9	----	----	----	6.0	5.9	5.6	----	----	----	5.5	5.8	----	6.1
	11	----	----	----	5.7	6.0	5.4	----	----	----	5.5	5.8	----	6.1
	14	----	----	----	5.6	5.6	5.5	----	----	----	5.4	5.8	----	----
	18	----	----	----	5.7	5.5	5.7	----	----	----	5.4	5.7	----	6.1
	22	----	----	----	5.6	5.5	5.5	----	----	----	5.4	5.6	----	6.1
Nov. 26, 1924:														
Saccharose (2 per cent), Difco peptone (0.2 per cent), distilled water	2	----	----	----	5.8	5.8	5.8	5.9	----	----	5.4	6.1	5.8	5.9
	3	----	----	----	5.7	5.6	5.6	5.8	----	----	4.9	5.8	5.7	5.8
	5	----	----	----	5.7	5.6	5.7	5.8	----	----	4.2	5.9	5.9	5.8
	8	----	----	----	5.9	5.8	5.6	5.8	----	----	4.2	5.8	5.9	----
	11	----	----	----	5.8	5.8	5.6	5.5	----	----	3.8	5.7	5.4	----
	14	----	----	----	5.5	5.6	5.0	4.7	----	----	3.6	5.5	5.7	5.8
	17	----	----	----	5.3	5.4	4.8	4.5	----	----	3.7	5.0	4.6	----
	22	----	----	----	4.2	5.1	4.1	4.3	----	----	3.8	5.2	4.2	5.9
	32	----	----	----	4.1	4.8	4.0	4.3	----	----	3.8	5.4	4.0	6.2
Nov. 26, 1924:														
Dextrose (2 per cent), Difco peptone (0.2 per cent), distilled water	2	----	----	----	5.5	5.7	5.4	5.5	----	----	4.8	5.4	5.6	5.6
	3	----	----	----	5.4	5.7	5.4	5.4	----	----	4.5	5.4	5.5	5.6
	5	----	----	----	5.4	6.0	5.1	5.5	----	----	4.1	5.4	5.4	5.6
	8	----	----	----	5.3	6.1	5.0	5.1	----	----	4.0	5.4	5.4	----
	11	----	----	----	4.1	5.6	4.4	4.2	----	----	3.6	5.0	4.8	----
	14	----	----	----	4.1	5.1	3.9	4.5	----	----	3.7	4.6	4.3	5.5
	17	----	----	----	4.1	4.6	4.2	3.9	----	----	3.6	4.6	3.9	----
	22	----	----	----	4.0	4.2	3.8	4.3	----	----	3.6	4.3	4.1	5.4
	32	----	----	----	4.0	4.0	3.8	4.2	----	----	3.6	4.2	4.2	5.7
Nov. 18, 1924:														
Dextrose (2 per cent), Difco peptone (0.2 per cent), in Altman at 18° C.	2	----	----	----	5.7	5.7	6.0	5.8	----	----	5.5	5.8	5.8	5.6
	4	----	----	----	5.8	5.7	6.0	5.8	----	----	5.6	5.8	5.8	----
	6	----	----	----	5.9	6.0	6.4	5.8	----	----	5.5	5.9	5.9	----
	8	----	----	----	5.8	5.9	6.4	5.9	----	----	5.2	5.9	5.9	----
	12	----	----	----	5.8	6.1	6.4	5.9	----	----	5.3	5.9	5.9	5.8
	16	----	----	----	5.7	5.9	6.4	5.9	----	----	4.8	5.9	5.9	----
Jan. 3, 1925:														
Dextrose (2 per cent), Difco peptone (0.2 per cent)	2	----	----	----	5.8	6.0	6.0	5.8	----	----	5.7	5.9	5.6	5.8
	3	----	----	----	5.8	6.1	6.2	5.9	----	----	5.5	5.9	5.0	----
	4	----	----	----	5.9	6.3	6.2	5.9	----	----	5.1	5.9	5.8	5.6
	6	----	----	----	6.4	6.5	6.3	5.9	----	----	5.2	5.8	5.0	----
	7	----	----	----	6.4	6.0	6.4	5.9	----	----	4.8	5.8	4.7	5.8

TABLE 3.—*Acid production, from different sugars in peptone solutions, by stripe-blight and halo-blight organisms, determined by the colorimetric method*—Contd.

Date inoculated and medium used	Incubation period (days)	P_H concentration of medium												
		After inoculation with stripe-blight organism isolation No.—							After inoculation with halo-blight organism isolation No.—					Uninoculated
		66b	69	70	9	10	11	12	Stock	57a	74	75	76	
Jan. 12, 1925:														
Dextrose (2 per cent), Difco peptone (0.2 per cent)	1 a. m.				6.1	6.1	6.1	6.2			5.7	6.0	6.2	5.9
	1 p. m.				5.8	5.9	6.0	5.9			5.8	5.9	5.9	
	2 a. m.				5.8	6.0	6.1	5.9			5.1	5.7	5.9	5.7
	2 p. m.				5.8	6.0	6.1	5.9			5.1	5.8	5.9	
	3 a. m.				5.9	6.0	6.2	5.8			5.2	5.7	5.6	
	3 p. m.				5.9	6.1	6.1	5.8			4.8	5.4	5.6	
	4 a. m.				6.0	5.8	6.0	5.8			4.2	5.4	5.3	5.7
	4 p. m.				5.9	5.9	6.0	5.8			4.3	5.1	5.7	
	5 a. m				5.9	5.9	6.3	5.8			4.5	5.2	5.9	
	7 a. m.				6.2	5.9	5.9	5.8			4.1	4.9	4.8	5.7
	7 p. m.				5.9	6.0	5.9	5.8			4.2	4.9	5.4	
	8 a. m.				6.0	5.9	5.8	5.8			4.1	5.0	6.2	
	9 a. m.				5.9	5.8	5.8	5.8			3.9	4.7	4.6	
	10 p. m.				6.2	5.8	5.6	5.8			4.0	4.8	4.5	
	11 p. m.				5.9	5.6	6.2	5.8			4.2	5.4	4.3	
	12 p. m.				6.4	5.8	5.8	5.9			4.0	5.4	4.5	
Feb. 2, 1925:														
Synthetic medium [a]	1				6.9	7.0	6.9	6.9			6.9	7.0	6.9	7.0
	2				6.8	6.9	6.9	6.9			6.8	6.8	6.8	
	3				6.8	6.8	6.7	6.7			6.7	6.7	6.7	
	4				6.7	6.7	6.6	6.7			6.6	6.5	6.6	7.1
	7				6.4	6.4	6.2	5.9			6.2	6.2	6.6	
Feb. 9, 1925:														
Dextrose (1 per cent), ammonium phosphate	1				6.8	6.8	6.8	6.8			6.7	6.8	6.8	6.9
	2				6.7	6.8	6.8	6.8			6.7	6.7	6.7	
	5				6.6	6.6	6.6	6.6			6.6	6.5	6.6	
	7				6.5	6.6	6.6	6.6			6.6	6.6	6.5	6.9
	8				6.4	6.5	6.5	6.5			6.5	6.5	6.4	
	9				6.2	6.3	6.5	6.4			6.4	6.5	6.4	
	10				6.3	6.3	6.4	6.4			6.2	6.5	6.5	
	12				6.2	6.2	6.3	6.5			5.9	6.4	6.3	
	15				5.8	5.9	5.8	6.0			5.9	6.3	6.2	6.9
	16				5.9	6.0	5.9	6.4			5.8	5.7	6.0	
	18				5.8	6.0	5.8	6.0			5.7	6.1	5.8	
	19				5.7	5.8	5.7	6.1			6.1	5.6	6.1	
	22				5.5	5.4	5.9	5.7			5.5	5.5	5.6	
	25				5.7	5.7	5.9	5.7			5.7	5.6	5.5	6.8
	33				5.2	5.2	5.5	5.7			5.6	5.4	5.2	6.8

[a] Synthetic medium, Manual of the Society of American Bacteriologists: Monobasic ammonium phosphate, 1 gm.; potassium chloride, 0.02 gm.; magnesium sulphate, 0.02 gm.; dextrose, 10 gm.; water, 1,000 c. c.

Table 3 gives the results of tests for acid production from saccharose, dextrose, and synthetic media, using the colorimetric method.

While in some instances the halo-blight organisms produced more acid than the stripe-blight organisms, the results as a whole show no consistent differences in quantities of acid produced.

TECHNICAL DESCRIPTION

Bacterium striafaciens n. sp.

A motile rod with rounded ends and polar flagella; single, in pairs, or in short chains; average measurements 0.66×1.76 μ; no spores; capsules formed; aerobic; white on agar; liquefies gelatin; does not produce indol; produces H_2S; forms a soft curd in milk, which is slowly peptonized; slight reduction of nitrates; starch hydrolyzed; ammonia produced; acid produced from dextrose, saccharose, and levulose, none from other carbon compounds tested; optimum temperature 22° C., thermal death point 48°; optimum reaction P_H 6.5 to P_H 7.0; Gram-negative; not acid fast; pathogenic on varieties of cultivated oats and to a slight degree on barley.

SUMMARY

A new bacterial disease of oats has been collected from the chief oat-growing sections of the United States. In the fields of oats observed by the writer the disease was not sufficiently abundant to make control measures necessary. Specimens from the Pacific coast, however, seem to be more heavily infected.

The lesions are water-soaked dots and streaks which later turn rusty brown. They show no halolike borders and under favorable conditions are covered with shining dried scales of exudate.

These stripe lesions are distinctly different from the lesions produced by *Bacterium coronafaciens* Elliott, *Bact. coronafaciens* var. *atropurpureum* Reddy and Godkin, and *Pseudomonas alboprecipitans* Rosen.

Repeated isolations have given a typical white organism which on inoculation produced typical stripe lesions quite different from the lesions of the halo-blight organism.

Inoculations of a large number of oat varieties have shown the same differences in varietal susceptibility to both stripe-blight and halo-blight organisms. The host range of the two organisms also was the same as far as tested.

No consistent differences in the reactions of the two organisms to various culture media have been detected.

Morphologically the stripe-blight organism is consistently smaller than *Bacterium coronafaciens*.

On the basis of the differences in type of lesions produced on oats and the smaller size of the stripe-blight organisms, the name *Bacterium striafaciens* n. sp. is suggested for the organism producing water-soaked dots and streaks on oats.

A COMPARATIVE STUDY OF THE QUINHYDRONE AND HYDROGEN ELECTRODES FOR DETERMINING THE HYDROGEN-ION CONCENTRATION OF SOILS [1]

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INTRODUCTION

The determination of the hydrogen-ion exponent of soils heretofore has been confined to two methods, the hydrogen electrode and the colorimetric. With the development of the quinhydrone electrode for practical use and its adaptation to soils by Biilmann (*3*)[2], extensive application of this electrode procedure has been made by virtue of its comparative simplicity and economy of time. In addition, when the presence of nitrates is sufficiently high to act as an interfering factor in the obtainment of constant potentials as a result of reduction by the hydrogen electrode, the quinhydrone electrode, as shown by Biilmann, may be used without danger of error from this source.

Results of hydrogen-ion exponent determinations on soils by the hydrogen and quinhydrone electrodes indicate excellent agreement as reported by some soil investigators. However, results to the contrary have also been reported. Christensen and Jensen (*8*) determined the P_H values of suspensions of many diverse Danish soils, ranging in P_H value from approximately 3.5 to 8.5, and found close agreement between the hydrogen and quinhydrone electrodes. Baver (*2*) reports that the hydrogen and quinhydrone electrode results compare closely, the variation ranging from 0 to 0.2 P_H. When working on soil suspensions Brioux and Pien (*7*), however, were unable to obtain in general the agreement between the two electrodes as reported by Christensen and Jensen. When centrifugates were used the agreement was better, although the quinhydrone results were slightly higher as a rule and there still remained cases of unexplainable deviations.

The present work was therefore undertaken to compare the two electrodes as used for the determination of the hydrogen-ion exponents of soil suspensions.

THEORETICAL CONSIDERATIONS

The familiar working formula for the computation of P_H values, using the hydrogen electrode is:

$$(1) \qquad P_H = \frac{E - E_{(cal.)}}{0.00019837\ T}$$

where—

E = observed potential.

$E_{(cal.)}$ = potential between calomel electrode and normal hydrogen electrode at t centigrade.

T = absolute temperature ($273.09 + t$ centigrade).

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[1] Received for publication Aug. 15, 1927; issued December, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 834.

Biilmann and his coworkers (*4, 5, 6*) determined the potential difference of cells of the type

$$Pt \mid \text{Quinhydrone, electrolyte} \mid H_2 \ (1 \text{ atm.}) \ Pt.$$

where the quinhydrone and hydrogen electrodes were in the same electrolytes of known P_H value, contact potential being eliminated by connecting the two electrodes with the common electrolyte. The measurements were made at 0°, 18°, 25°, and 37° C., respectively, and it was found that within the limits of experimental accuracy, the potential-temperature curve of the quinhydrone electrode over this range is practically a straight line represented by the following equation:

$$\pi_t = 0.7175 - 0.00074\, t \tag{2}$$

where the potential of the electrode at $t°$ and 760 mm. dry hydrogen is represented by π_t, the quinhydrone electrode being positive to the hydrogen electrode. The largest difference between the calculated and observed potentials was found to be 0.4 millivolts. By a simple substitution in which E in equation (1) is replaced by $\pi_t - E_q$ where E_q is the observed potential difference between the quinhydrone and calomel electrodes, the working equation for the quinhydrone electrode becomes:

$$P_H = \frac{\pi_t - E_{(cal.)} - E_q}{0.00019837\ T} \tag{3}$$

The standard calomel electrode may be replaced by a standard quinhydrone electrode as suggested by Veibel (*16*). However, in the present work the saturated calomel electrode was used by preference and assigned the $E_{(cal.)}$ values given by Clark (*9*).

The quinhydrone electrode results are calculated from equation (3), where the π_t and $E_{(cal.)}$ values are referred to a hydrogen partial pressure of 760 mm.

When employing either the saturated, normal, or tenth-normal calomel electrodes there is a current reversal at a definite P_H value depending upon the electrode used and the temperature. From equation (3) it is obvious that with the tenth-normal calomel electrode at 18° C. the potential becomes zero at about P_H 6.34 and negative with increasing P_H. With the saturated calomel electrode at 18° this change of potential from positive to negative occurs at about P_H 7.86. With those soils below P_H 7.86 the observed potentials when using the saturated calomel electrode will be positive, since the quinhydrone electrode will be positive to the calomel electrode, and above P_H 7.86 the potentials will be negative.

The sign of the observed potential difference is that of the quinhydrone electrode in relation to the known sign of the standard calomel electrode.

EXPERIMENTAL WORK

QUINHYDRONE CONCENTRATION

After a few preliminary experiments in which the quinhydrone concentration was varied from about 0.02 gm. to approximately 0.5 gm., using the soil-water ratio of 1 to 2, it was concluded that about 0.1 gm. of quinhydrone was sufficient for soil suspensions.

Three grades of quinhydrone were used, one a high-grade purchased product m. p. 169°–170° and the remaining two prepared in the laboratory by the method of Biilmann and Lund (*6*). Results with the three preparations were in agreement.

Introduction of the quinhydrone to the soil suspension was facilitated by means of a glass spoon constructed so as to deliver about 100 mgm. of quinhydrone.

Baver (*2*) used in his work on the quinhydrone electrode 0.05 gm. of quinhydrone per 15 c. c. of solution, and Hissink and Van der Spek (*13*) 0.05 gm. for 5 gm. of soil and 20 c. c. of water.

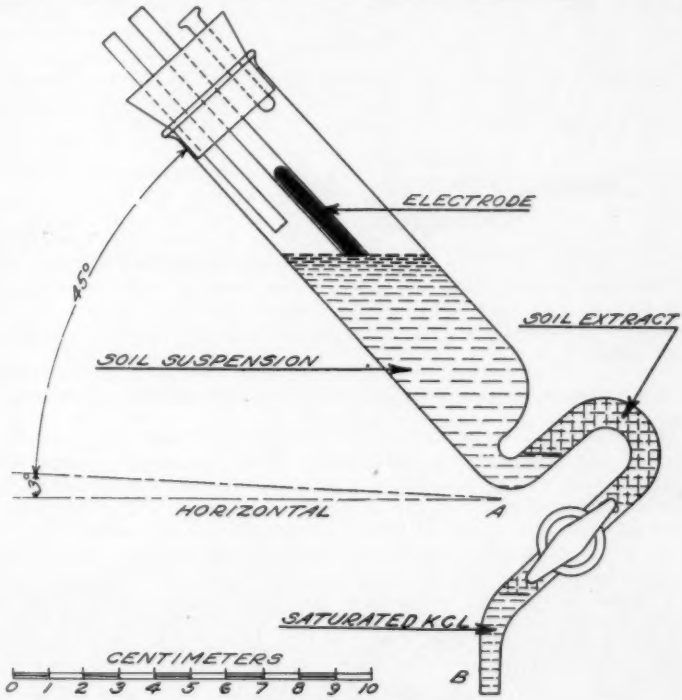

FIG. 1.—The electrode vessel

SOIL-WATER RATIO

All measurements, electrometric and colorimetric, were made on soil suspensions with a soil-water ratio of 1 to 2, using 15 gm. of air-dried soil (passing a 2 mm. sieve) and 30 c. c. of water. This mixture was shaken by hand in a large pyrex test tube for approximately 1 minute and allowed to stand about 20 minutes for sedimentation.

The colorimetric measurements were made on the centrifugate of the supernatant liquid, employing Gillespie's drop-ratio method (*11*), with the substitution of bromcresol green (*14*) for methyl red.

ELECTRODE VESSEL

The electrode vessel of pyrex glass shown in Figure 1 is a closed type, being a modification of the one employed by Gillespie (*10*),

and was used for both quinhydrone and hydrogen electrode measurements. The volume of the vessel is about 65 c. c., the ratio of the volume to that of the soil suspension being such as to insure thorough mixing during shaking.

The application to soils of the principle of shaking the electrode vessel with alternate exposure and immersion of the electrode, especially in hydrogen electrode measurements, allows of a more rapid attainment of equilibrium between gaseous hydrogen, the electrode, and the soil suspension. A localized equilibrium with this procedure is avoided, and one is obtained which is more characteristic of the entire suspension.

ELECTRODE

The electrode shown in Figure 2 consists of a sheet of platinum foil about 2.5 by 3.0 cm. securely held in place by two platinum leads for the current, which were welded to both ends of the foil and sealed into the glass support. The electrodes, while occasionally heated to redness in the alcoholic flame, as suggested by Biilmann (*3*) for the quinhydrone electrode procedure, were also frequently treated with hot chromic-acid solution and when not in use were kept in a chromic-acid bath. The gold-foil electrodes were about 1.0 by 2.0 cm. and were welded to a short piece of platinum wire and then sealed into a glass tube.

FIG. 2.—The electrode

VARIATION OF QUINHYDRONE ELECTRODE MEASUREMENTS WITH TIME

To determine the variation of the quinhydrone electrode with time, different soil types were investigated. Potential measurements were made on suspensions of duplicate samples of these soils immediately after an initial shaking of the electrode vessel and then after definite intervals with the vessel at rest. The potential differences given in Table 1 are characteristic of several duplicate measurements made on each sample and are typical of a large number of results.

A large number of potentials measured immediately after an initial shaking of about 30 seconds and then after the following 1, 5, and 10 minute intervals, with the vessel at rest, were similar to those shown in Table 1.

In Table 1 the initial time of shaking the electrode vessel has been increased to 2 minutes, and the potentials read immediately after shaking and after the following intervals indicated. In those instances where there was a final shaking of the electrode vessel for 2 minutes, little further change in the potentials was observed. The constancy of potential of the acid soils may in general be considered good over the 15-minute period, although there was a small potential decrease or corresponding P_H increase with time.

With the alkaline soils the potentials maintained as a rule a good degree of constancy over the 15-minute period, in some instances as high as about P_H 9.0. There were, however, occasional exceptions to this on both the acid and alkaline sides. Quinhydrone electrode measurements on one black alkali soil of P_H 9.0 (hydrogen electrode value) drifted rapidly over a 15-minute period, the initial quinhydrone

value being about P_H 9.5. In instances of this nature the drift was such that any measurement is admittedly a "forced" one.

TABLE 1.—*Variation of quinhydrone electrode measurements with time*

Soil type	Electrode vessel shaken 2 minutes		Electrode vessel at rest						Electrode vessel shaken 2 minutes	
			5 minutes		10 minutes		15 minutes			
	Volts	P_H	*Volts*	P_H	*Volts*	P_H	*Volts*	P_H	*Volts*	P_H
Norfolk fine sandy loam, Norfolk, Va.	0.1729	4.73	0.1712	4.76	0.1704	4.77	0.1699	4.78	0.1693	4.79
	.1734	4.72	.1722	4.74	.1711	4.76	.1707	4.77	.1700	4.78
Caribou loam, Aroostook County, Me.	.1706	4.82	.1678	4.87	.1668	4.88	.1658	4.90	.1651	4.91
	.1679	4.86	.1656	4.90	.1644	4.92	.1643	4.93	.1640	4.93
Washburn loam, Aroostook County, Me.	.1230	5.47	.1209	5.50	.1184	5.54	.1180	5.55	.1176	5.56
	.1214	5.50	.1195	5.54	.1187	5.55	.1174	5.57	.1176	5.57
Portsmouth fine sandy loam, New Bern, N. C.	.1196	5.60	.1194	5.60	.1187	5.62	.1185	5.62	.1182	5.62
	.1198	5.60	.1189	5.61	.1175	5.64	.1173	5.64	.1170	5.64
Miami silty clay loam, Wells County, Ind.	.0151	7.40	.0160	7.39	.0160	7.39	.0169	7.37	------	------
	.0128	7.44	.0126	7.44	.0128	7.44	.0139	7.42	------	------
Stockton clay adobe, Butte County, Calif.	−.0456	8.41	−.0522	8.52	−.0542	8.56	−.0547	8.57	−.0548	8.57
	−.0448	8.40	−.0501	8.49	−.0560	8.59	−.0562	8.59	−.0547	8.57
Prowers clay loam, Lamar, Colo.	−.0434	8.36	−.0431	8.35	−.0428	8.35	−.0425	8.34	−.0405	8.31
	−.0425	8.34	−.0431	8.35	−.0433	8.36	−.0430	8.35	−.0411	8.32
Fort Lyon clay loam, Las Animas, Colo.	−.0347	8.26	−.0390	8.33	−.0390	8.33	−.0386	8.32	−.0370	8.30
	−.0374	8.30	−.0387	8.33	−.0386	8.32	−.0386	8.32	−.0364	8.29
Minnequa silty clay loam, Rocky Ford, Colo.	−.0646	8.73	−.0671	8.77	−.0673	8.78	−.0666	8.77	−.0631	8.71
	−.0639	8.72	−.0651	8.74	−.0656	8.75	−.0656	8.75	−.0620	8.69
Fallon loam, Churchill County, Nev.	−.0852	9.20	−.0905	9.29	−.0905	9.29	−.0930	9.33	−.0910	9.30
	−.0860	9.21	−.0878	9.24	−.0893	9.27	−.0897	9.28	−.0877	9.24

Quinhydrone electrode measurements were also made at intervals over a 10-minute period, with the electrode vessel shaking continuously as in the procedure recommended by Gillespie (*10*) for hydrogen electrode determinations. The potentials were in general similar to those recorded in Table 1, in which instance the electrode vessel was not shaken continuously.

As a result of many observations it seems advisable to make the potential measurement about one-half to one minute after the addition of the quinhydrone.

QUINHYDRONE ELECTRODE PROCEDURE

The procedure adopted for the determination of the P_H values of soils with the quinhydrone electrode is as follows: Fifteen grams of air-dried soil and 30 c. c. of water are shaken in a large pyrex test tube approximately one minute. This suspension is allowed to stand a short time for sedimentation, and a portion of the supernatant liquid sufficient to fill the neck of the vessel and bore of the stopcock is poured into the electrode vessel. (Fig. 1.) The end *B* is filled with saturated potassium-chloride solution and the vessel attached to a shaker, the actuating arm of which passes through the axis *A*. The end *B* is then connected to the saturated calomel electrode. The remainder of the soil suspension is poured into the vessel and about 0.1 gm. of quinhydrone added. The plain platinum electrode (fig. 2) is placed in the electrode vessel, which is shaken one-half to one minute. The potential is then read immediately with the vessel at rest.

Contact with the calomel electrode is drawn into the end *B*, thus preventing the entrance of potassium chloride into the soil suspension. The potential is measured with a Leeds & Northrup type K potentiometer in connection with a high-grade galvanometer and a Bureau

of Standards certified Weston cell. The recorded temperature, which was that of a small room especially adapted to potentiometric work, did not vary greatly during the day; however, temperature corrections were applied to all measurements.

HYDROGEN ELECTRODE PROCEDURE

The procedure followed for the hydrogen electrode determination is as follows: The soil treatment and filling of the electrode vessel are as described for the quinhydrone electrode procedure prior to adding the quinhydrone. The electrode (fig. 2) was lightly coated with palladium in about 5 seconds, electrolyzed in dilute sulphuric acid, thoroughly washed in distilled water, then placed in the vessel (fig. 1), and the space above the soil suspension thoroughly swept out with pure hydrogen, but not bubbled through the suspension. During shaking, which is at the rate of about 80 complete swings per minute, contact with the calomel electrode is drawn into the end *B* and the potential read after 5-minute intervals, the second reading being a control. Equilibrium is generally obtained in 5 to 10 minutes.

VARIATION OF HYDROGEN ELECTRODE MEASUREMENTS WITH TIME

The hydrogen electrode measurements shown in Table 2 were made after the indicated periods, during and after shaking of the electrode vessel.

TABLE 2.—*Variation of hydrogen electrode measurements with time*

Soil type	Electrode vessel shaking						Electrode vessel at rest					
	5 minutes		10 minutes		15 minutes		1 minute		5 minutes		10 minutes		15 minutes	
	Volts	P_H	*Volts*	P_H	*Volts*	P_H	*Volts*	P_H	*Volts*	P_H	*Volts*	P_H	*Volts*	P_H
Norfolk fine sandy loam,	0.5279	4.75	0.5290	4.77	0.5293	4.78	0.5298	4.79	0.5298	4.79	0.5298	4.79	0.5298	4.79
Norfolk, Va.	.5283	4.76	.5283	4.76	.5286	4.77	.5290	4.77	.5291	4.77	.5291	4.77	.5291	4.77
Caribou loam, Aroostook	.5296	4.75	.5311	4.77	.5298	4.75	.5323	4.79	.5330	4.80	.5328	4.80	.5329	4.80
County, Me.	.5307	4.79	.5312	4.79	.5307	4.79	.5335	4.83	.5342	4.84	.5346	4.85	.5351	4.86
Washburn loam, Aroostook	.5709	5.45	.5699	5.43	.5699	5.43	.5721	5.47	.5728	5.48	.5737	5.49	.5741	5.50
County, Me.	.5715	5.46	.5702	5.44	.5694	5.42	.5725	5.47	.5736	5.49	.5743	5.50	.5746	5.51
Portsmouth fine sandy	.5844	5.68	.5831	5.66	.5830	5.66	.5835	5.66	.5832	5.66	.5832	5.66	.5832	5.66
loam, New Bern, N. C.	.5823	5.64	.5814	5.63	.5818	5.64	.5818	5.64	.5818	5.64	.5818	5.64	.5818	5.64
Miami silty clay loam,	.6902	7.49	.6877	7.45	.6858	7.42	.6903	7.49	.6899	7.49	.6894	7.48	.6882	7.46
Wells County, Ind.	.6873	7.44	.6870	7.44	.6861	7.42	.6884	7.46	.6883	7.46	.6883	7.46	.6883	7.46
Stockton clay adobe, Butte	.7137	7.91	.7145	7.93	.7138	7.91	.7170	7.97	.7174	7.99	.7161	7.95	.7160	7.95
County, Calif.	.7124	7.89	.7116	7.88	.7116	7.88	.7146	7.93	.7154	7.94	.7146	7.93	.7147	7.93
Prowers clay loam, Lamar,	.7186	7.97	.7189	7.98	.7185	7.97	.7195	7.99	.7197	7.99	.7198	7.99	.7200	7.99
Colo.	.7185	7.97	.7183	7.97	.7178	7.96	.7194	7.98	.7196	7.99	.7196	7.99	.7197	7.99
Fort Lyon clay loam, Las	.7200	8.01	.7187	7.99	.7183	7.98	.7210	8.03	.7209	8.03	.7207	8.02	.7207	8.02
Animas, Colo.	.7200	8.01	.7183	7.98	.7177	7.97	.7203	8.02	.7204	8.02	.7204	8.02	.7203	8.02
Minnequa silty clay loam,	.7509	8.52	.7496	8.50	.7489	8.49	.7502	8.51	.7504	8.52	.7509	8.52	.7509	8.52
Rocky Ford, Colo.	.7482	8.48	.7478	8.47	.7475	8.47	.7503	8.51	.7510	8.53	.7513	8.53	.7513	8.53
Fallon loam, Churchill	.7911	9.17	.7912	9.17	.7917	9.18	.7942	9.22	.7944	9.23	.7941	9.22	.7938	9.22
County, Nev.	.7912	9.17	.7891	9.14	.7890	9.14	.7926	9.20	.7930	9.20	.7918	9.18	.7920	9.19

The results indicate in general the reproducibility and constancy that may be obtained, and are quite representative of several thousand determinations made during the last eight years, being exclusive, however, of those occasional instances of erratic potentials referred to below.

Gillespie and Hurst (*12*) have given a frequency table showing the number of times potential changes were observed due to 5 minutes' extra shaking. The readings were made during shaking after 5-

minute intervals, the second reading being a control. As a result of 58 determinations the mean potential drop due to the extra 5 minutes' shaking was 0.9 millivolt. It is stated by these authors that "if the electrode vessel is not shaken during the measurement the potential is not constant, but becomes numerically greater, sometimes so fast that any measurement would be a forced one." This, they say, is due to the presence of nitrates and possibly to other oxidizing agents. With palladium black electrodes a similar potential rise after bringing the electrode vessel to rest has been observed, particularly with alkaline soils when using impure hydrogen containing small quantities of oxygen. Under similar conditions, electrodes of platinum or iridium black on platinum or gold foil showed insignificant potential variations.

It has been observed, as further stated by Gillespie and Hurst (*12*), that "if the vessel were not shaken during the measurements, and a final potential reading were taken after the potential seemed to become constant, the results in some cases would be quite erroneous, and it would appear that in general such results would be more or less in error." The results given in Table 2 seem to bear out Gillespie's statement of the constancy of potential during shaking of the electrode vessel.

As a rule the reproducibility of hydrogen electrode measurements on soil suspensions has been within about 3 millivolts. It should be remembered, however, that the hydrogen electrode is not universally applicable, especially in the presence of unsaturated substances and active oxidizing agents, and there may be soils containing constituents which would cause unsatisfactory results and preclude the use of the hydrogen electrode. In instances of this nature, which may occur occasionally, or where the investigator has reason to suspect the validity [3] of his results, or when there is difficulty in obtaining stable and reproducible potentials with the hydrogen electrode, it is advisable to compare the hydrogen electrode, the quinhydrone electrode, and the colorimetric methods with one another.

PALLADIUM, PLATINUM, AND IRIDIUM BLACK ELECTRODES

Experiments with hydrogen electrodes of palladium, platinum, or iridium black on platinum or gold foil showed that, under equilibrium conditions, results on soil suspensions by these different electrodes are in close agreement.

Palladium, platinum, and iridium black were lightly deposited in about 5 seconds from approximately 3 per cent solutions of palladium chloride, chloroplatinic acid, and iridium sesquichloride, respectively, each containing about 2 per cent of hydrochloric acid.

In regard to plating electrodes it might be well to mention at this point that it does not seem improbable that the properties of the plating solution as well as the current density employed are factors contributory to the character of the deposit. As stated by Clark (*9*), "since there is a simultaneous deposit of metal and hydrogen and,

[3] A soil sample submitted to the writer gave with a hydrogen electrode a negative potential and a correspondingly absurd P_H value for a soil. It was noticed that the box in which the soil had been sent was labeled "Bichloride of Mercury." The treatment of 15 gm. of a similar sandy soil low in organic matter with as little as 10 mgm. of $HgCl_2$ produced an effect greater than that mentioned above, this apparently being an instance of electrode poisoning. The effect on other soils higher in organic matter was comparatively small. A second sample of the original soil, but free from $HgCl_2$, was found to have a P_H value of 5.3.

since the character of the platinum, palladium, or iridium black is undoubtedly due to the vigor of the hydrogen evolution, it is evident that the P_H of the solution constitutes a part of the conditions." In this connection Andrews (*1*) states that "palladium electrodes for hydrogen-ion determinations are much less reliable than platinum electrodes because of the lack of permanence of the former which results from a more or less rapid change of amorphous to crystalline palladium."

COMPARISON OF RESULTS

Some of the results characteristic of those obtained by using the two procedures outlined for the quinhydrone and hydrogen electrodes are shown in Tables 3 and 4, together with a few available colorimetric results.

TABLE 3.—*Comparison of P_H results on soils*

Hydrogen electrode	Quinhydrone electrode	Hydrogen electrode	Quinhydrone electrode	Hydrogen electrode	Quinhydrone electrode	Colorimetric	Hydrogen electrode	Quinhydrone electrode	Colorimetric
P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
3.92	4.10	5.41	5.48	7.48	7.42	7.5	8.13	8.34	7.9
4.20	4.13	5.47	5.50	7.54	7.87	7.6	8.14	8.16	7.9
4.26	4.40	5.53	5.65	7.60	7.77	7.5	8.20	8.00	8.1
4.52	4.47	5.61	5.76	7.61	7.79	7.6	8.20	8.35	7.9
4.53	4.48	5.62	5.67	7.64	7.85	7.7	8.20	8.49	7.9
4.78	4.75	5.80	5.75	7.72	8.00	7.5	8.21	8.38	8.0
4.81	4.88	6.00	6.05	7.82	8.31	7.7	8.31	8.53	8.1
4.90	4.80	6.00	6.20	8.00	8.20	8.0	8.36	8.37	8.2
4.93	4.98	6.00	6.30	8.00	8.35	8.0	8.51	8.61	8.5
5.00	5.20	6.09	6.00	8.00	8.40	8.0	8.62	8.60	8.5
5.04	5.00	6.20	6.40	8.04	8.34	7.9	8.64	8.79	Turbid.
5.07	4.90	6.64	6.80	8.07	8.24	8.1	8.85	8.96	Turbid.
5.28	5.17	6.80	7.14	8.10	8.30	8.0	9.00	9.50	Turbid.
5.36	5.66	7.46	7.50	8.10	8.30	7.8	9.17	9.20	Turbid.

The agreement between the two methods as shown in Table 3 may, with the exception of a few values around P_H 8.0, be considered good for soils, particularly on the acid side. On the alkaline side the larger variations were more frequent, although instances of good agreement were obtained as high as P_H 9.2. In those instances where the colorimetric results are given, there is in general better agreement with the hydrogen than with the quinhydrone electrode results, the quinhydrone electrode in these instances apparently being in error.

La Mer and Parsons (*15*) found that the quinhydrone electrode gave reliable results in buffered solutions more acid than P_H 8.0, provided no rapid oxidizing or reducing agents were present. Erroneous results obtained in more alkaline solutions are attributed to the autoxidation of hydroquinone.

In an article by Biilmann (*4*) there is discussed the influence on the potential of chemical compounds present in the quinhydrone electrode, such as the acidity of the hydroquinone, the dissociation and the influence of acids and salts on the activities of hydroquinone and quinone. It is also stated by this author (and this point should be kept in mind) that "the quinhydrone electrode is by no means a universal electrode, and there may be soils containing constituents which affect the electrode potential."

TABLE 4.—*Comparison of P_H results on buffer solutions*

Electrode	P_H values							
Hydrogen	1.50	2.90	4.58	7.49	7.98	8.25	8.59	8.94
Quinhydrone	1.53	3.02	4.56	7.48	7.96	8.20	8.55	8.90

The hydrogen and quinhydrone electrode results given in Table 4 for some of Clark and Lubs's (*9*) buffer solutions ranging from about P_H 3.0 to 8.0 are in excellent argeement, the largest deviation between the two electrodes being 0.03 P_H. Above P_H 8.0 the quinhydrone electrode potentials were not so constant but continued to drift over a 15-minute period. From P_H 8.0 to about 9.0, as far as these investigations were carried, readings made prior to any appreciable drift; that is, about 1 minute after the addition of quinhydrone, gave results agreeing closely with those of the hydrogen electrode. At about P_H 9.0 the drift of the quinhydrone electrode potentials over a period of 15 minutes had risen to about 6 millivolts.

Many quinhydrone electrode potentials obtained with platinum and gold-foil electrodes on some of Clark and Lubs's buffer solutions ranging from about P_H 2.3 to 8.0 showed that the potentials obtained with gold-foil electrodes at intervals of ½, 1, 5, and 10 minutes after the addition of quinhydrone were more nearly constant than those observed with platinum electrodes. With gold electrodes as a rule a constant oxidation-reduction potential was quickly established, whereas with platinum electrodes there was generally a slight drift toward more negative potentials. The P_H values obtained with gold quinhydrone electrodes were as a rule in closer agreement with the hydrogen electrode values than those obtained with platinum quinhydrone electrodes.

SUMMARY

Many results on soils from about P_H 4.0 to 9.0 indicate that the constancy of the quinhydrone electrode measurements may, with an occasional exception, be considered good.

Hydrogen electrode measurements of acid and alkaline soils from about P_H 4.0 to 9.0 made on soil suspensions, using a soil-water ratio of 1 to 2, were on the whole reproducible and constant. Equilibrium was generally obtained in 5 to 10 minutes.

Results on soil suspensions with hydrogen electrodes of palladium, platinum, or iridium black on platinum or gold foil were under equilibrium conditions in close agreement.

Results on soil suspensions from about P_H 4.0 to 9.0 by the hydrogen and quinhydrone electrode methods showed on the whole good agreement for soils, particularly on the acid side. On the alkaline side the larger variations were more frequent.

There was excellent agreement between results obtained by the quinhydrone and hydrogen electrode methods on acid and alkaline buffer solutions up to about P_H 8.0. From about P_H 8.0 to 9.0—as high as the investigations were carried—the quinhydrone electrode potentials drifted gradually.

Results obtained on buffer solutions with the quinhydrone electrode showed that gold-foil electrodes were preferable to platinum.

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NODULAR LESIONS IN THE SPLEEN OF SWINE CAUSED BY ACTINOMYCES INFECTION[1]

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ACTINOMYCOSIS IN DOMESTIC ANIMALS

Actinomycosis is a well-known, chronic, infectious disease of rather common occurrence in cattle, but is encountered less frequently in swine and other domestic animals. The infective agent, *Actinomyces bovis*, or ray fungus, may gain entrance through injuries of mucous membranes, particularly of the mouth or through abrasions of the skin. The lesions are usually localized but may become generalized.

In hogs the primary lesions are usually found in either the tonsils or the mammary glands. Occasionally the infection gains entrance through castration wounds of pigs.

Recorded cases of actinomycosis of swine involving the spleen or other internal organs are not very numerous. Zietzschmann (*8*)[2] describes a generalized case but does not state whether the spleen was affected. Knoll (*5*) observed a case in a pig involving the lungs, liver, and other tissues, but apparently no splenic lesions were noted. Carl (*2*) mentioned a case of actinomycosis in an 8-month-old pig in which the lungs, liver, stomach, and spleen were affected. Among a number of cases reported in swine by Assmann (*1*) there was one animal which showed lesions of the mammary glands, lungs, and spleen.

SOURCE OF MATERIAL

For a number of years veterinary inspectors of the meat-inspection service, Bureau of Animal Industry, while making routine postmortem inspections at slaughtering establishments, have found from time to time isolated cases of nodular lesions of the spleens of swine in which no lesions were observed in other organs or tissues. This apparent absence of lesions in the carcass, other than those observed in the spleen, and the fact that the lesions simulate somewhat those of tuberculosis, have served to stimulate rather unusual interest in these cases.

After making a histological examination of a number of such nodular spleens, received at the pathological laboratory for diagnosis, the writer found in sections from one of them a number of small, red-staining bodies showing morphological characteristics of *Actinomyces bovis*, or ray fungus.

Following these findings a special effort was made to obtain as many specimens of the nodular spleens as possible from hogs showing no other lesions, in order to determine the frequency of occurrence of the actinomycoticlike bodies in the nodules and their possible relation from an etiological standpoint.

[1] Received for publication July 26, 1927; issued December, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 841.

It was the intention at first to make only a histological study of the spleens, but after examining a number of specimens, several of which showed bodies simulating actinomyces, it was decided also to culture a number of the spleens in the hope that a bacteriological examination would aid somewhat in establishing more definitely the causative factor in these cases.

The observations recorded in this paper have extended over a period of several years, during which at least 50 typical specimens of the nodular spleens have been received at this laboratory. A number of the specimens were forwarded for diagnosis in routine manner from various bureau stations; others were obtained by request through inspectors in charge at the large hog-slaughtering establishments. Most of the specimens were examined histologically but only about one-half of them were cultured.

GROSS APPEARANCE OF THE NODULAR SPLEENS

The nodules in the different spleens vary considerably in size. Some are little larger than the head of a pin, while others may attain the size of a hazelnut. More frequently they are about the size of a pea or a little larger. The more superficial nodules were usually slightly elevated above the surface of the organ.

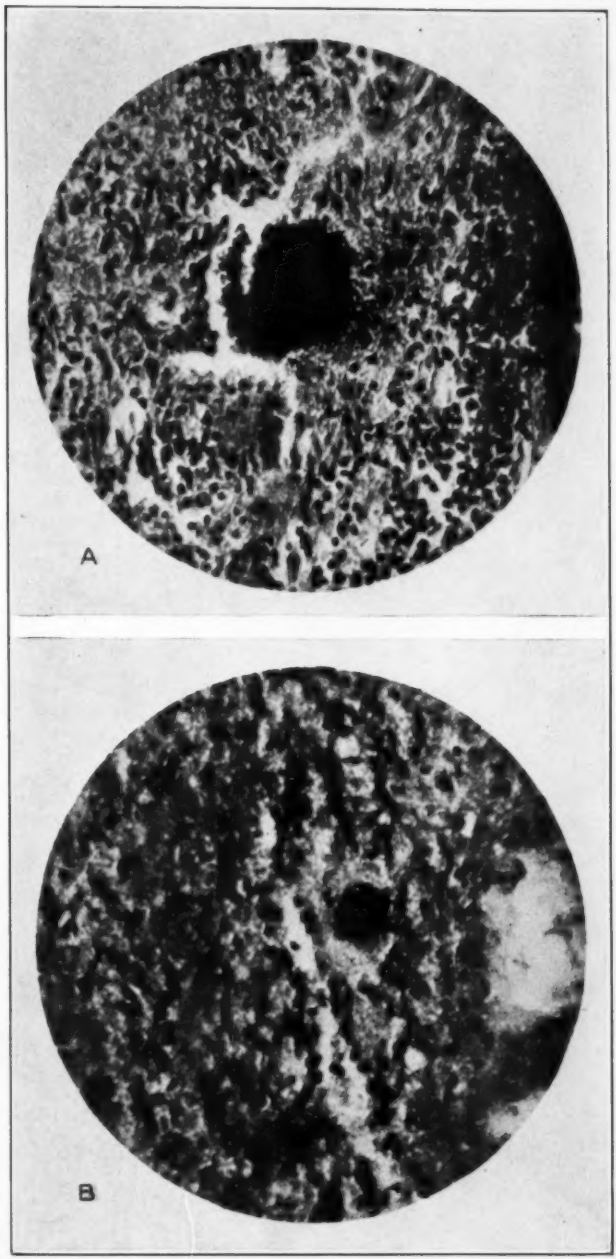
While on casual observation the nodular lesions in the gross specimen are somewhat suggestive of tuberculosis, a more minute examination reveals a rather distinct difference. The spleen does not show the extensive fibrous changes seen in tuberculosis. The nodules consist of a homogeneous material heavily encapsulated. The necrotic centers are of a cheeselike consistence in the younger lesions, and are usually of a greenish or greenish-yellow color. Frequently these necrotic masses can be readily detached and removed from the surrounding capsule. There is a tendency to calcification in the older lesions. The splenic pulp usually shows no visible changes outside the encapsulated nodules.

HISTOPATHOLOGY

Sections from affected spleens show the lesions to consist of necrotic centers, varying in size and usually heavily encapsulated. The necrotic centers are surrounded by zones of round cells, epitheloid cells, and leucocytes. The degenerated centers of the nodules are frequently seen to be undergoing calcification. In most cases little change was noted in the splenic tissue outside of the encapsulated areas.

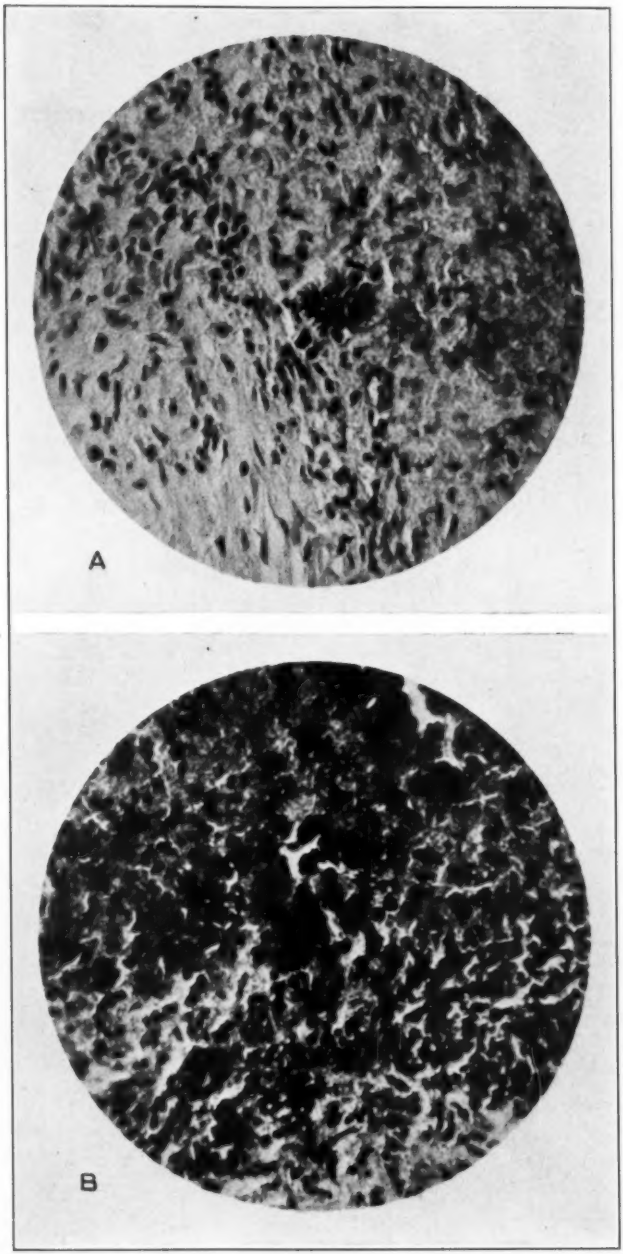
The actinomyces have been definitely demonstrated in seven different specimens, or 14 per cent of the spleens examined. (See pls. 1 to 5.) In other spleens degenerated bodies were also seen which were thought to be actinomyces, but they could not be definitely recognized as such on account of the poor staining resulting from degenerative changes. The undegenerated colonies, or those which had undergone only slight degenerative changes, were found to be positive to Gram's stain.

When the red-staining bodies were first observed in the swine spleens it was noted that they did not appear to be altogether typical of *Actinomyces bovis*. It is now believed that the slight morpho-

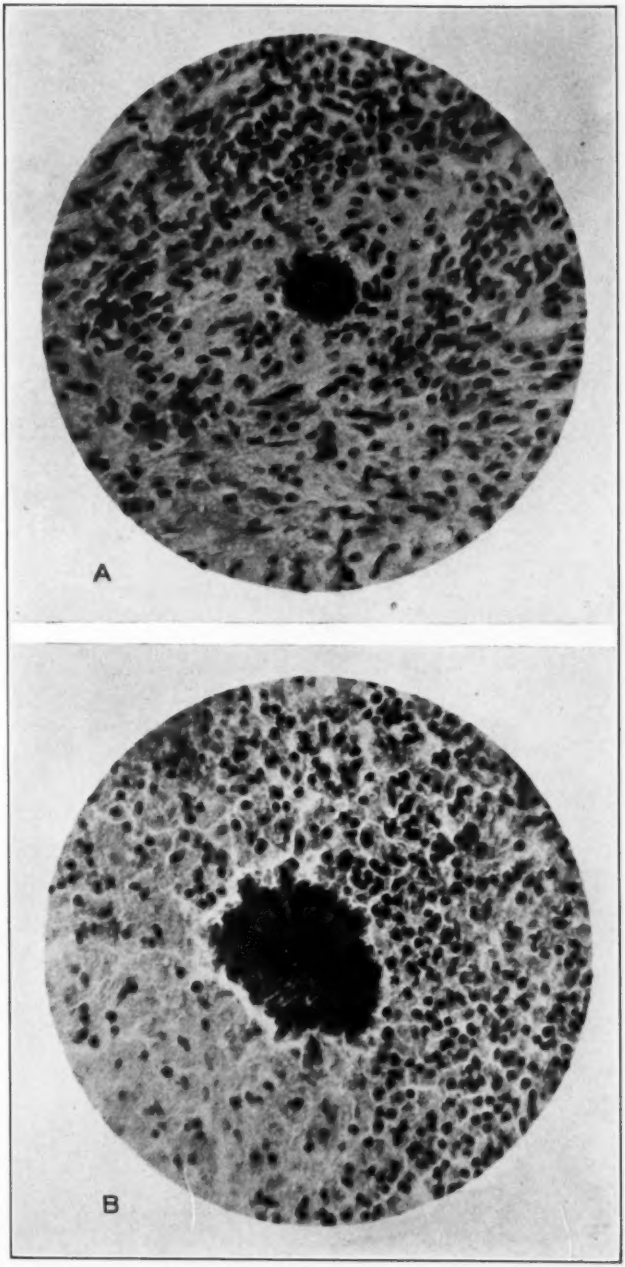


A.—Photomicrograph of section of spleen, case 5-D, showing the only colony of the organism found in this specimen. × 400

B.—Photomicrograph of section of spleen, case 6-D. A number of small bodies similar to the one here shown were found in this case. × 400

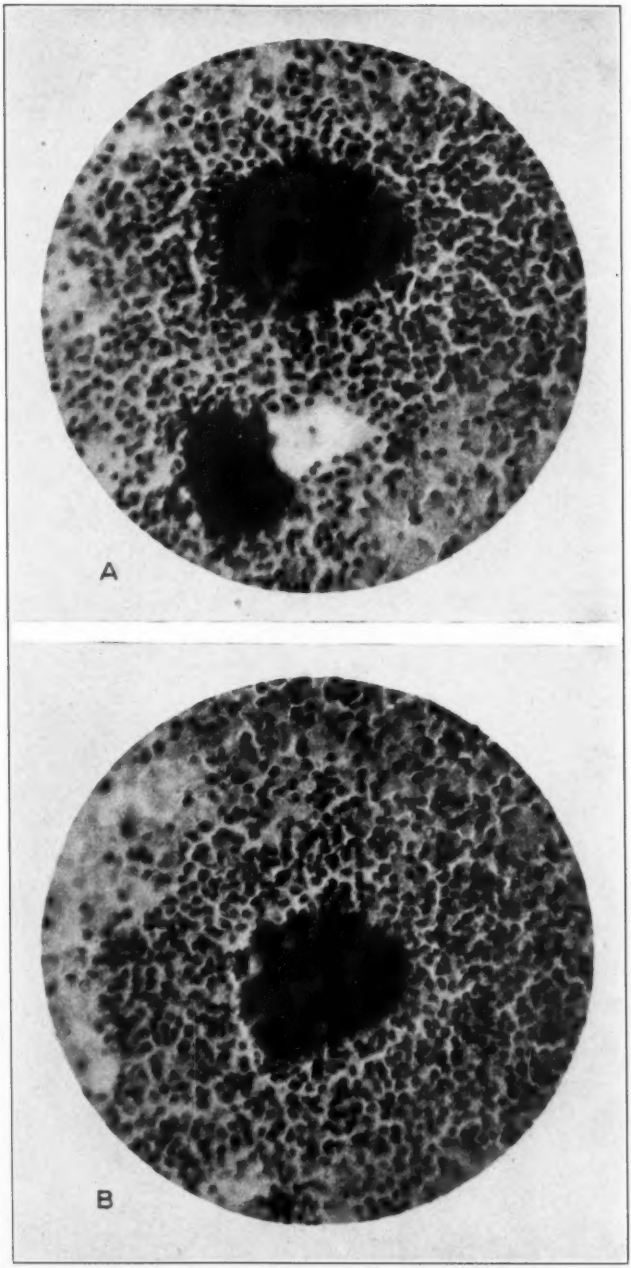


A.—Photomicrograph of section of spleen, case 9–D. The small colony here shown was the only one found in the various sections examined. × 400
B.—Photomicrograph of section of spleen, case 7–F, showing the only colony that could be definitely recognized in this case. × 400

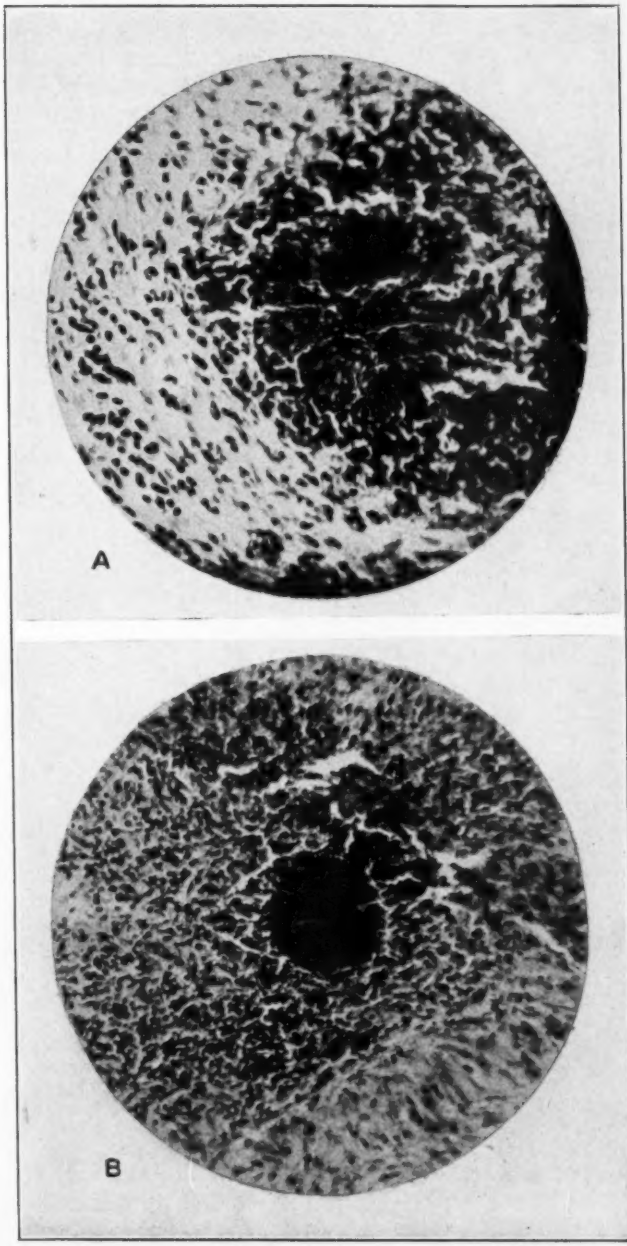


A.—Photomicrograph of section of spleen, case 8-F, showing the only colony found in this case. × 400

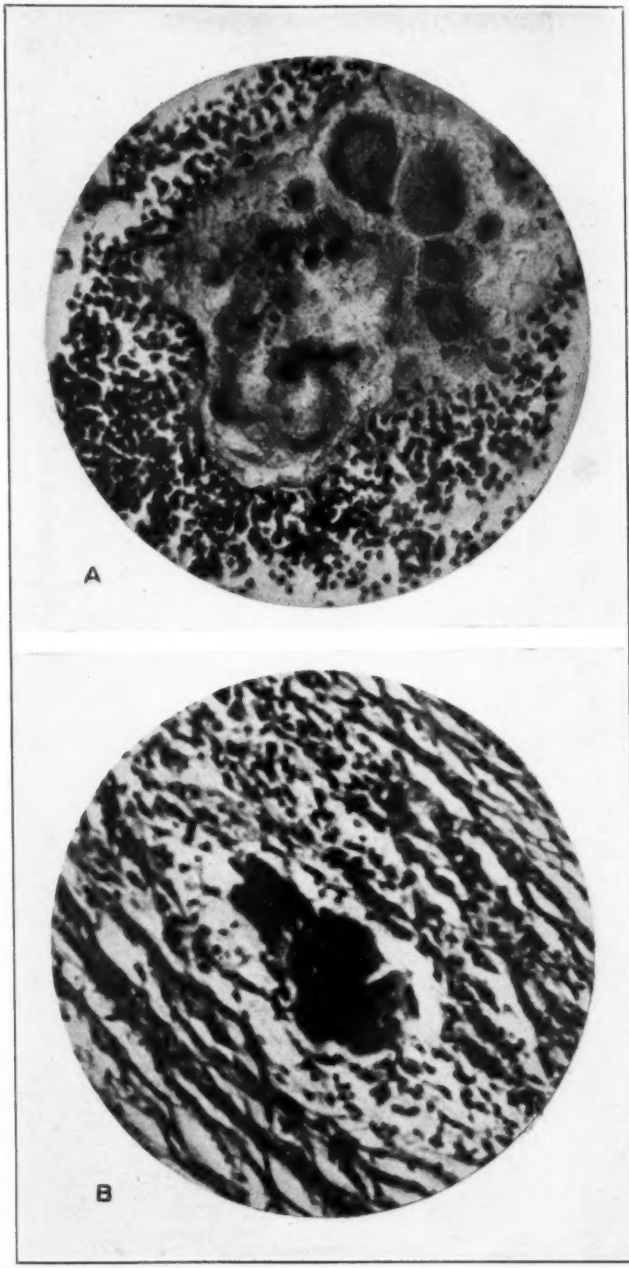
B.—Photomicrograph of section of spleen, case 9-F. A considerable number of colonies quite characteristic of *Actinomyces bovis* were found in this specimen. × 400



A and B.—Photomicrographs of sections of spleen, also from case 9-F, showing two more of the rather typical colonies found in the different nodules in this specimen. × 400



A and B.—Photomicrographs of sections of spleen, case 2-R. Practically every nodule in this spleen contained one or more colonies. × 350 and × 250, respectively



A.—Photomicrograph of section of stomach, case 13–D, received from L. E. Day, showing colonies of the organism in lesions of stomach wall. × 400
B.—Photomicrograph of section of stomach, case 1–P, showing a partially degenerated colony in center of the stomach wall. × 350

logical variations noted were the result of degenerative changes, as somewhat similar alterations have been observed in the organisms in cases of actinomyces infection of bovine tissues. Doubtless there had been more or less degeneration of the actinomyces even in those cases in which they were definitely recognized, and this is thought to account largely for the fact that growth of the organism was obtained in only one of the various specimens cultured.

In order to show the marked similarity of the pathological changes in the nodular spleens the following brief description is given of the lesions found in a number of the specimens, which are more or less typical of all.

CASE 3–D.—In this case there were several rather large necrotic areas surrounded by zones of leucocytic and round-cell infiltration, the whole being encapsulated. There were also a few small necrotic foci. No changes were observed in the splenic pulp outside of the encapsulated areas.

CASE 4–D.—Sections show a few small, necrotic foci which were rather heavily encapsulated and undergoing calcification. Accumulations of round cells and lecucocytes are seen within the capsules, and in several places there were fusions of epithelioid cells simulating giant-cell formation.

CASE 5–D.—Nodules showed large necrotic centers surrounded by zones of cells beneath the heavy capsules. There is considerable calcification of the nodules. In one of the sections a small, rather well-defined body, quite suggestive of actinomyces, was observed, being the only one found in this case. (See pl. 1, A.)

CASE 6–D.—Nodules with necrotic centers showing borders of round cells inclosed in thick capsule. Sections from this specimen show several of the actinomycoticlike bodies, mostly within the necrotic areas. There were also a number of degenerated and partially degenerated colonies in the different sections examined. (See pl. 1, B.)

CASE 9–D.—Sections from this specimen show a number of small necrotic foci massed together and surrounded by a fibrous wall. Excessive numbers of eosinophiles were noted in the cellular infiltrations. At the border of one of the necrotic areas one of the small bodies simulating actinomyces was noted. (See pl. 2, A.)

CASE 11–D.—Practically all the nodules in this case were undergoing calcification. Within the encapsulated areas were numerous degenerated and partially calcified bodies which in size and outline were rather suggestive of actinomyces. A few of these bodies took the eosin stain but were not clearly enough defined to be identified.

CASE 1–F.—Lesions consisted of a number of small, thickly encapsuled nodules. Practically the entire encapsulated contents of the various nodules had undergone calcification. There were no changes in the spleen outside of the encapsulated areas.

CASE 3–F.—In this specimen there were a few large and a number of small nodules in different stages of degeneration and calcification, all well encapsulated.

CASE 7–F.—Each of the sections in this case contained one fair-sized nodule, encapsulated, and showed the usual necrotic and calcified centers. In one of the sections a rather characteristic actinomycoticlike body was observed. A partially degenerated colony was also noted in one other section. (See pl. 2, B.)

CASE 8–F.—Lesions consist of the typical necrotic centers with the surrounding cellular infiltrations and encapsulated. In the various sections in this case only one of the small bodies simulating actinomyces was found. (See pl. 3, A.)

CASE 9–F.—Spleen showed nodular lesions throughout. The character of the lesions was similar to those already described. A considerable number of colonies of actinomyces were seen in this case. Some of the colonies were rather large and in varying stages of degeneration. (See pl. 3, B, and pl. 4, A and B.)

CASE 2–R.—Sections from this spleen showed only a few large nodules. The nodules are well encapsulated and exhibit the typical necrotic centers. Numerous eosinophiles were seen in the cellular zones surrounding the degenerated centers. Practically every nodule in this specimen contained one or more colonies of actinomyces. (See pl. 5, A and B.)

CULTURAL RESULTS

Some of the spleens received for culturing were in a fair state of preservation, while others, on account of post-mortem changes, gave unsatisfactory results. It may be stated in this connection that the cultural results might have been more satisfactory had the material been fresh in all cases.

The method of procedure used in culturing spleens was as follows: A number of the nodules were removed from the spleen in a sterile manner and ground in a sterile mortar. The ground material was then mixed with a small amount of sterile normal salt solution. After melted agar had been cooled to about 45° C. a small amount of blood serum was added. The agar was then inoculated with the splenic emulsion and plated. Agar slants, plain bouillon tubes, and Smith tubes containing liver bouillon were also inoculated with the material. The inoculated culture media were incubated under both aerobic and anaerobic conditions.

In a considerable number of the cases cultured the media remained sterile. In others there was an overgrowth of contaminating microorganisms. In one instance, however, a few colonies were obtained of an organism which in its morphology and in its cultural and staining characteristics proved to be quite typical of actinomyces. The organism grew poorly at first on agar slants but gave an abundant growth after it had become fully accustomed to aerobic conditions.

ANIMAL INOCULATIONS

GUINEA PIGS AND RABBITS.—One guinea pig was inoculated subcutaneously with 1 c. c. of a macerated agar culture of the actinomyces; two were inoculated intra-abdominally with 1 c. c. each of the agar culture; and two were fed 1 c. c. each of a mixture of the agar and bouillon cultures of the organism.

One rabbit was inoculated intravenously with 2 c. c. of a bouillon culture of the actinomyces; another was inoculated intra-abdominally with 2 c. c. of a macerated agar culture; and a third was given 2 c. c. of the agar culture subcutaneously.

SWINE.—One pig between 2 and 3 months of age was inoculated intra-abdominally with 10 c. c. of a bouillon culture of the actinomyces. Another pig about the same age received intravenously 10

c. c. of a bouillon culture of the organism. Each of three pigs about 3 months of age was fed several hundred cubic centimeters of a mixture of bouillon and agar-slant cultures of the actinomyces mixed with ground feed.

All the guinea pigs, fed and inoculated, gave negative results.

Two of the rabbits, inoculated intra-abdominally and intravenously, respectively, showed no evidenee of actinomycotic infection when slaughtered seven weeks after inoculation. The third rabbit, which was inoculated subcutaneously, showed a small abscess at the point of inoculation several weeks after receiving the injection. When the animal was slaughtered the abscess was found to contain an inspissated pus. No actinomyces could be demonstrated microscopically in the pus, and culture inoculations showed the abscess to be sterile.

The swine which were fed and inoculated with the cultures of actinomyces were slaughtered at intervals varying from two to six months following the feedings and inoculations. Post-mortem examinations of the animals failed to show any evidence of actinomycotic infection of any of the organs or tissues.

Since it was especially desired to infect swine with the actinomyces, and the negative findings in the experimental pigs seemed to indicate the nonvirulence of the culture isolated, no further attempts were made to infect animals with this strain of the organism.

The character of the lesions in the nodular spleens and, in many cases, the apparent absence of living actinomyces indicate that the affected pigs are in most instances successful in overcoming the infection. It seems probable that this apparent resistance to the infection also results in a reduced virulence of the causative organisms obtained in cultures from such lesions. This may account, partially at least, for the writer's inability to infect pigs with the culture obtained from the nodular splenic lesions. It is a well-known fact, however, that it is a difficult matter to infect animals with cultures of actinomyces, or at least to produce lesions typical of those resulting from natural infection.

Warwick (*7*) makes the statement that investigators have found it difficult to produce experimental actinomycosis by inoculation. In fact it appears that comparatively few workers have been successful in producing the disease with cultures of actinomyces. Lignière and Spitze produced actinomycosis in cattle with cultures of bovine origin. It appears, however, that their material was obtained from an unusually virulent form of actinomycosis, which had assumed an epizootic character in Argentina. Their work does not seem to have been recorded in the literature, and the opinion has been expressed by some investigators that this was probably an outbreak of actinobacillosis rather than actinomycosis.

Magnusson (*6*) was successful in producing actinomycosis in cattle by culture inoculations, and he makes the statement that at the time his work was reported, in 1923, he knew of no other European investigator who had obtained positive results from similar inoculations. He is of the opinion that the sporadic nature of the disease in Europe tends to decrease its virulence. If his deduction relative to the decreased virulence of the disease is correct it would hold equally true in this country, where the disease is also usually of a sporadic nature.

Therefore, after taking into consideration the difficulties experienced by others in their attempts to produce actinomycosis by culture inoculations, it is not altogether surprising to find the inoculation results here reported negative.

POSSIBLE MODE OF INFECTION

The fact that the lesions in practically all of these cases appear to be confined to the spleen has given rise to considerable speculation as to the manner in which the infective agent finds its way to that organ. Herzog (*4*) is of the opinion that the ray fungus is spread by way of the lymphatic circulation. If we consider the close proximity of the spleen and stomach, and the lymphatic relation of these two organs, it does not appear unreasonable to assume that the infection in these cases first finds lodgment in the stomach, either by simple absorption or actual infection of the stomach wall, and eventually reaches the spleen either by contiguity or through the lymphatic circulation.

During the time that these observations were being made two cases of actinomycotic infection of the stomach of swine came to the writer's attention. One of these was diagnosed by L. E. Day at the branch pathological laboratory, Chicago, Ill., and the other was received for diagnosis at the pathological laboratory, Washington. In both instances the stomach wall was very much thickened and contained fibrous nodules, one of the cases showing necrotic and calcified centers.

In the first-mentioned case the red-staining fungus growths can be readily seen in the lesions. (Pl. 6, A.) In the other the bodies are very much degenerated. (Pl. 6, B.) In both instances the lesions involve practically the entire stomach wall in places.

While the writer has no knowledge that the spleen was involved in either of these cases of stomach infection, there is of course the possibility that there might have been slight lesions in the deeper structures of the spleen which could not be readily detected in the macroscopical examination. However, regardless of such a possibility, the demonstration of these cases of infection of stomach wall strengthens the theory that the most probable way in which the infection may reach the spleen is through the stomach.

It is likewise true that in some of the animals showing nodular spleens there may have been associated stomach lesions which were slight in nature, rendering them inconspicuous on post-mortem examination. Such lesions being quite unusual might easily have been overlooked by the inspector making the post-mortem inspection.

Feldman (*3*) reports a case of actinomycotic infection of the omentum and peritoneum of a cow in which all other organs and tissues were free from the disease. He was at a loss to account for the source of entry of the causative organism. This case is mentioned simply to illustrate how the actinomyces may reach an internal organ or tissue without leaving any visible trace to indicate its path.

APPARENT RESISTANCE OF AFFECTED ANIMALS

As a large percentage of hogs are slaughtered before reaching 1 year of age, most of the specimens of nodular spleens examined were obtained from animals ranging from 6 to 12 months of age. The

chronic character of the lesions indicates that the pigs probably become infected during the first few months of life. The resistance of the animal to this splenic type of infection is evidenced by the heavy fibrous wall thrown around the infected centers, and the sterile condition of the encapsulated necrotic material in a large percentage of cases. This would account for the presence of the actinomyces in only a small percentage of the cases examined, and as many of the colonies seen in sections were found to be undergoing degenerative changes it probably also accounts for the fact that cultures of the organism could be obtained from only one of the various specimens cultured. In other words, it would seem that the finding of the living or undegenerated organisms in these cases is rather difficult and depends largely on the age of the lesions at the time the hog is slaughtered.

CONCLUSIONS

The similarity of the lesions, both macroscopically and microscopically, in the cases investigated is indicative of a common etiological factor.

The fact that no other possible causative factor was found in the lesions, and that *Actinomyces bovis* was recognized in at least seven different specimens, seems sufficient to identify this organism as the cause of the splenic lesions.

The character of the lesions and the large percentage of cases in which the causative organism was no longer present indicate that the pigs probably become infected early in life, and that they are quite resistant to such splenic infections.

The evidence at hand points to the stomach as the most probable portal of entry through which the actinomyces eventually reach the spleen.

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A MODIFICATION OF BRUCE'S METHOD OF PREPARING TIMBER-YIELD TABLES [1] [2]

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In Bruce's method of preparing timber-yield tables [2] anamorphosis is used for constructing the curves for site classification, total basal area, average basal area, number of trees, and total cubic volume. Since, by construction, each set of curves is represented by a series of straight lines radiating from a common point, it is evident that the reading of any two lines must have the same ratio at each age. Any curve of such a series can therefore be expressed as a percentage of any other curve of that series, such as the average curve. This attribute of anamorphically constructed series of curves [3] permits their expression in the form of alignment charts, the use of which eliminates the laborious anamorphic plotting of the data and the balancing of a curve for each site-index [4] class, results in increased accuracy, and makes interpolation and checking easier.

The first step in constructing these alignment charts is the preparation, for site classification, of an average curve [5] over age for—

(1) Height of average dominant tree;

and, for the entire stand, similar curves for—

(2) Total basal area, hereafter called stand basal area.
(3) Average basal area, hereafter called tree basal area.
(4) Number of trees.
(5) Volume in cubic feet.

These curves are similar to the graduating curves used by Bruce, but may be drawn in the conventional way rather than as graduating curves. The average points for the tree basal area curve should be computed from the averages of stand basal area and the numbers of trees used for constructing the average curves for these two factors, instead of using averages of tree basal areas of individual plots; otherwise the final curves for tree basal area will not balance.

The curves for tree basal area, numbers of trees, and stand basal area are checked against each other; at any age the product of tree basal area and number of trees should equal the stand basal area.

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[1] Received for publication June 25, 1927; issued December, 1927.

[2] BRUCE, D. A METHOD OF PREPARING TIMBER-YIELD TABLES. Jour. Agr. Research 32:543–557, illus. 1926. Errata: Figures 7 and 8 should be interchanged and the applied stand graph should be referred to Figure 7 instead of Figure 8.

[3] This applies, of course, only to systems of curves having a common origin.

[4] "Site index" is the height which the average dominant tree will have, or had, at some standard age, usually 50 (or 100) years.

The site-index system should not be considered as a banding system comparable with but more finely subdivided than the ordinary "I, II, III" or "Good, medium, poor" system. Rather, the farmers' classification of farm land as 45-bushel wheat land (in 1 year implied), or 24-bushel corn land (1 year) is entirely comparable with 93-foot (50-year) longleaf pine land. The only difference is that farm-land quality is rated by actual yield, but forest-land quality, because of the variation in yield with density of stocking, must be rated by measurement of some other factor (height) indicative of the productive capacity, but for the most part not appreciably affected by variations in density.

The grouping of plots into 10-foot site-index classes is done only for convenience in preparing the curves, and in the final tables values are given for only the even 10 feet of site index to avoid bulkiness. Values for every foot of site index, or even smaller intervals, could be determined, but intervals less than 1 foot are not practicable.

[5] These curves should be carefully balanced so that the sums of the positive and negative deviations are equal and distributed as evenly as possible throughout the length of the curve.

In addition to the curves listed above a so-called average *forest form factor-age* curve is drawn for checking purposes. This "forest form factor" is obtained by dividing the cubic volume by the product of stand basal area and the dominant heights [6] used for the site classification (*height-age*) curve.

Age-class averages of cubic volume, height, and stand basal area should be used to derive the average forest form factor for each class instead of computing the form factor for each plot and then averaging the form factors by age classes. The curve thus derived is used to check the stand basal area, height, and cubic volume curves against each other. At any age the volume should equal the product of stand basal area, height of the average dominant tree, and forest form factor.

A site classification alignment chart is now prepared from the *height-age* curve and a percentage alignment chart. Percentage charts may have either arithmetic or logarithmic scales. The logarithmic type gives better intersections and readings of the same relative accuracy throughout and is also less cumbersome to use. For these reasons logarithmic charts should be employed.

To construct the percentage chart lay out three parallel axes, A, B, and C (fig. 1), B midway of the others. On A and C lay out equal logarithmic scales. On B lay out a logarithmic scale with cycles half as large as those used on A and B, so placed that the 100 on this scale lies exactly on the line through 100 on A and C. (Only the B axis need be actually graduated, as will be shown later.) A line passing through any two numbers on A and B will intersect C at the point representing the ratio or percentage $\frac{B}{A} \times 100$.

To convert this chart into the site classification chart all that is necessary is to let the graduations on the A and B axes represent the height of the average dominant tree in feet, and to superpose on A and C the scales for age and site index, as follows:

On the A axis the ages are marked opposite the graduations corresponding to heights read from the average *height-age* curve, as listed below, resulting in scale A.

Age in years	Height of average dominant tree in feet	Age in years	Height of average dominant tree in feet
10	14	60	78
15	26	70	83
20	37	80	87
30	53	90	90
40	64	100	92
50	71		

Each site index expressed as a percentage of the average site index [7] is then marked opposite that percentage on the C axis, resulting in scale C'. This may also be done graphically by drawing, from the classification age on A, a series of lines to C passing through the 10-foot intervals on B. Their intersections on C fix the positions of the 10-foot site-index graduations. The intermediate site-index graduations may be located in the same way. The unnecessary

[6] The dominant height is substituted for average height to permit checking the site-index curves with the volume and stand basal area curves. Curves for average height are not constructed by anamorphosis and are therefore not susceptible to such simple checking.

[7] This is the height of the average *height-age* curve at the classification age (50, 100, etc., years).

logarithmic (height and percentage) graduations on A and C can now be erased, leaving only the age graduations on A', the heights on B', and the site indices on C'. A line through the age of a plot

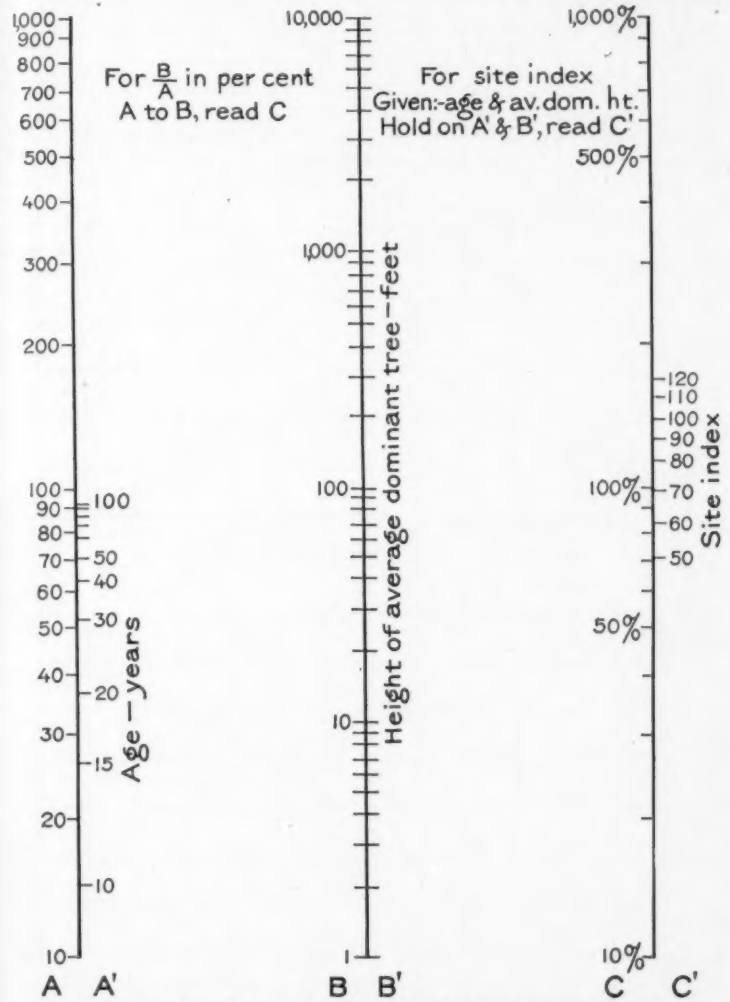

FIG. 1.—Logarithmic chart for computing percentages, converted into chart for classifying site

and the height of its average dominant tree will then intersect C' at the site index of that plot.

The next step in the use of this chart is the assignment of a site-index value to each plot, after which the plots are grouped by site-index classes (10-foot classes preferably).

Working first with stand basal area, an estimated stand basal area corresponding to the age of the plot is read from the average *stand basal area-age* curve. (Fig. 2, A.) Both observed and estimated

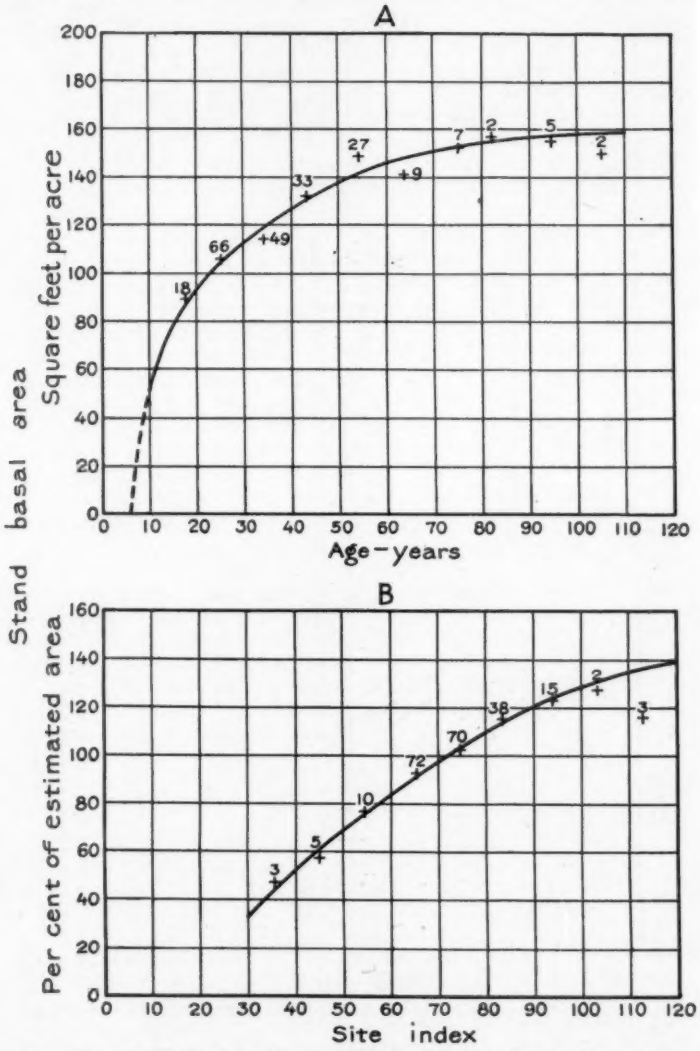

FIG. 2.—Curves used for preparing alignment chart stand basal area: A, average curve over age, used to graduate age axis in Figure 3; B, average curve over site index, used to graduate site-index axis in Figure 3

areas are then totalled by site-index classes and the sums of the observed values expressed as percentages of the sums of the estimated. Table 1 illustrates this. These percentages are than plotted over

the average site index of the class and curved, as in Figure 2, B. Similar curves are prepared for tree basal area, number of trees, and cubic volume, and are checked against each other. For each site index the percentage of stand basal area should equal the product of the percentage of number of trees and percentage of tree basal area.[8] The cubic volume curve could be checked through forest form factor, but ordinarily such a check is not needed, since the *volume per cent-site index* curve is usually a well-defined and straight or nearly straight line. Readings from the curve for stand basal area are then superposed on a percentage alinement chart, as was done for the site classification chart; the ages as obtained from the average *stand basal area-age* curve appearing on the *A* axis, and the site indices, from the *stand basal area per cent-site index* curve, appearing on the *C* axis. Similar charts are derived for tree basal area and diameter, number of trees, and total volume of peeled stem wood in cubic feet.

TABLE 1.—*Computations of stand basal areas, by site index classes, as percentages of stand basal area of average site index*

Plot No.	Number of plots	Site index	Age	Stand basal area		
				Observed	Estimated from average curve over age	Observed in per cent of estimated
			Years	*Sq. ft.*	*Sq. ft.*	*Per cent*
8		31	16	36	83	
46		36	24	56	102	
31		39	21	42	97	
Total	3	106		134	282	
Average		35.3				47.5
112		40	32	77	116	
80		41	27	46	108	
168		49	44	110	133	
119		47	34	48	119	
25		48	20	48	94	
Total	5	225		329	570	
Average		45.0				57.7
55		50	25	51	104	
153		57	40	86	128	
Total *	10	545		935	1,223	
Average		54.5				76.5

SUMMARY

3	35.3		134	282	47.5
5	45.0		329	570	57.7
10	54.5		935	1,223	76.5
72	65.2		8,183	8,835	92.6
70	74.1		8,894	8,715	102.1
38	83.4		5,354	4,620	115.9
15	94.0		1,937	1,573	123.1
2	103.0		232	182	127.5
3	113.0		311	268	116.0
Total 218			26,309	26,268	

* Totals are for 10 plots in this group, of which only 2 are shown.

[8] If the average curves over age have not been perfectly balanced these curves will not pass through 100 per cent at the average site index. Thus a simple check is combined with an automatic correction for any inaccuracies in balancing the curves drawn in the first steps.

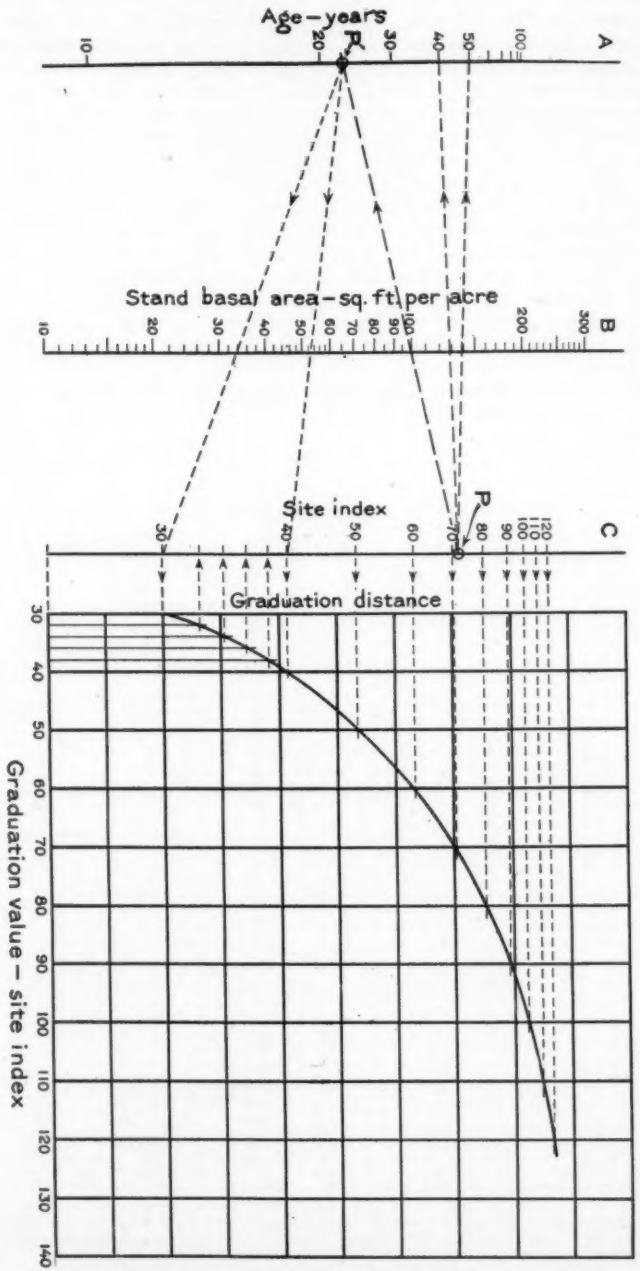

FIG. 3.—Alignment chart for stand basal area, indicating the location of the major graduations by intersection, and the preparation and use of a graduating curve for locating minor graduations for site index

To avoid the necessity for first constructing percentage charts, a somewhat different procedure may be followed. The three parallel axes are first drawn, not necessarily at uniform intervals. On the central axis a logarithmic scale of stand basal area is entered, as in Figure 3. Any point P is then temporarily marked on C as the position of the average site-index graduation. Lines drawn from P and intersecting B at values read from the average *stand basal area-age* curve will intersect A at the location of the corresponding age graduations.

For locating the site index graduation on C the graduations of B are temporarily considered as percentages, and a line from P, through 100 per cent, locates a point P' on A. Lines from P', intersecting B at percentages, read from the *stand basal area per cent site-index* curve, will intersect C at the position of the corresponding site-index graduations.

Charts for each of the other factors are similarly constructed. The chart for tree basal area can be read directly in terms of average breast-high diameters by marking the diameters along the B scale where the corresponding basal areas appear, as in Figure 4. Percentages for tree basal area should be computed from totals of stand basal area and number of trees by site-index classes.

This chart for diameters is also used for obtaining partial-stand values in terms of percentages of the corresponding total-stand values. The curves described by Bruce [9] are used for this.

A point (P, fig. 4) is permanently marked to the right of the A' axis. A series of axes parallel to X are drawn (B, C, D, etc.), their number depending on the number of ratios to be represented. Percentages are then marked on these axes at their intersection with lines, from P, through the corresponding average breast-high diameters of the total stand on X'. The same procedure is followed for the average diameters of the partial stands, except that the curves used by Bruce (difference between partial and total stand diameters over total stand diameter) are replaced by curves of partial-stand diameters over the entire-stand diameter.

Only a few graduations on the axis of any of the alignment charts need be located by intersection. The distances of these from any fixed point on the axis are then curved over the value of the graduation (age, site, index, diameter, etc.) and the distances of the intermediate graduations transferred from this curve. Figure 3 illustrates the location of the intermediate site-index graduations from the curve passing through the distances of the 10-foot graduations.

When the aggregate checks of the various factors are made it will be necessary in the case of tree basal area to weight the values for each plot by the observed number of trees; this is equivalent to comparing the observed stand basal area with that obtained by multiplying estimated tree basal area by the observed number of trees.

By Bruce's method the fit of the radiants indicates whether or not anamorphosis can be legitimately employed in preparing the curves. A consistent failure of the points to fall into straight lines indicates that the method is not suitable.

With the modifications here presented the applicability of anamorphosis can be tested as follows: The observed values and the estimated

[9] Bruce, D. Op. cit. p. 549, fig. 5; p. 551, fig. 6.

values corresponding to the age and site index of each plot are grouped, summed, and averaged by 10-year age—10-foot site-index classes. The deviations of the observed averages from the estimated aver-

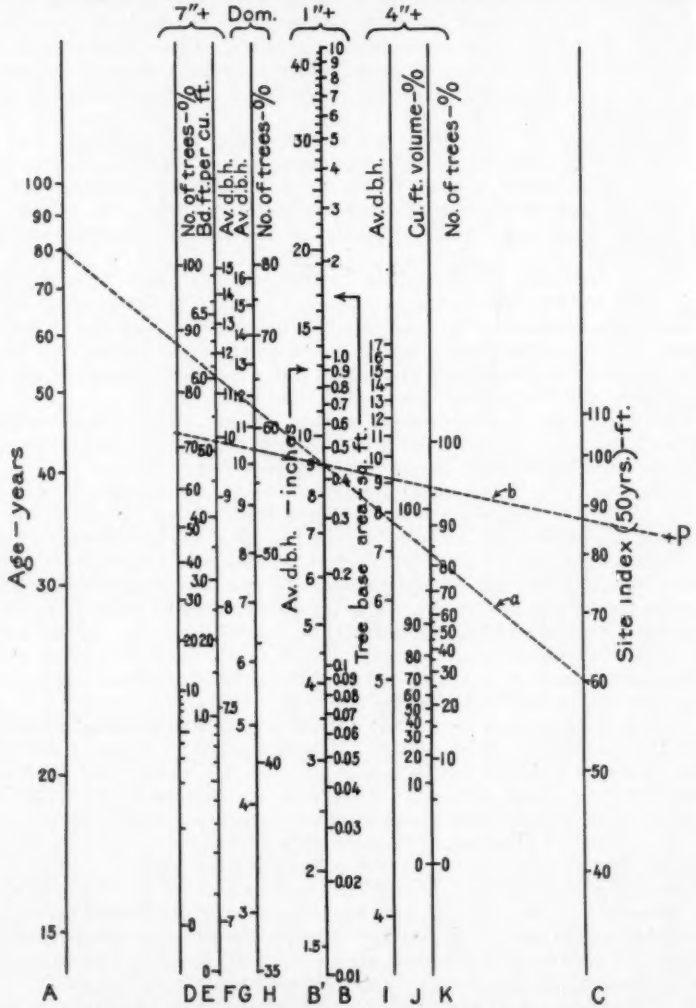

FIG. 4.—Alignment chart for tree basal area with scale reading in terms of average d. b. h. (*B'*). The additional axes, *D* to *K*, are graduated to show the partial stand values in per cent of the total stand values when a straight line is passed through *P* and the average d. b. h. of the total stand

ages are plotted over age by site-index classes. Failure of the method to fit will be indicated by a series of progressively changing curves. This is not the case with stand basal area in the illustrative example as shown in Figure 5. Such a test, so essential in the development

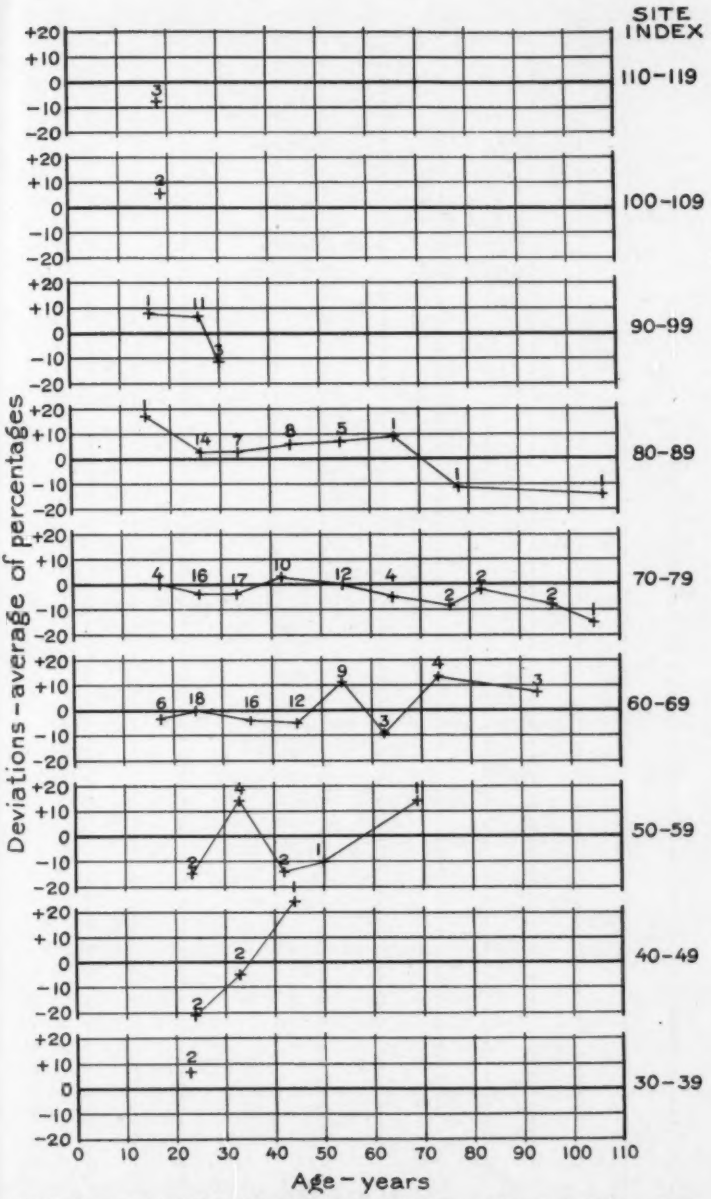

FIG. 5.—Percentage deviations of stand basal area plotted over age, by 10-foot site-index classes, showing that no consistent departure from the chart values exists, thus proving that the method used introduces no distortion

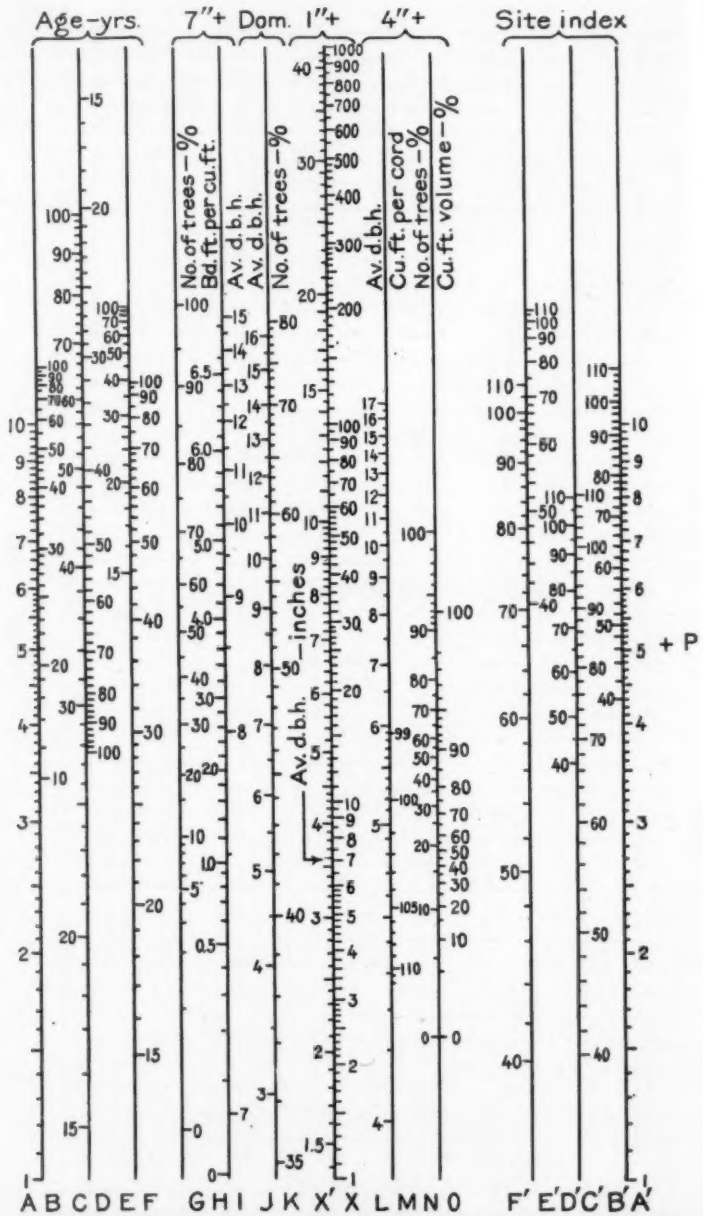

FIG. 6.—Composite chart for total-stand and partial-stand values. All total-stand values are read on the central scale. The two outer scales, *A* and *A'*, are used with *X* for multiplying total-stand values by partial-stand percentages to get partial-stand values. (See appendix for instructions for using this chart

of a new technic, is of little value now. Experience with nine species of diversified characteristics has justified the assumptions basic to the method.

Separate charts need not be made for each factor; they may be combined into a single chart by using a common central axis for all factors and separate outside axes for each factor, as shown in Figure 6. In this case the readings on the central logarithmic scale are multiplied or divided by 10 or 100, as necessary for the factor considered.

An additional pair of scales used in conjunction with the central

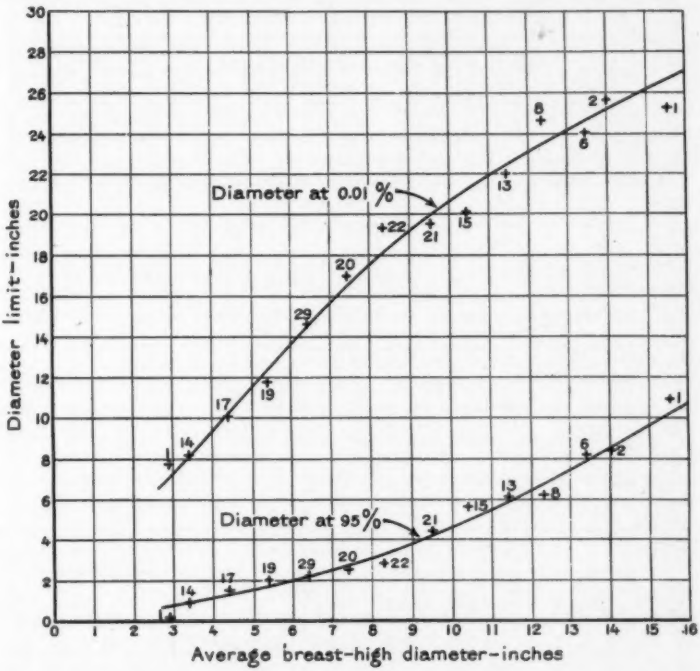

FIG. 7.—Intercept curves for stand-table graph. The diameters at which the basic frequency curves intercept the 95 and 0.01 ordinates are plotted over the average diameters (by basal area) of the respective frequency curves. The curves fitted to these points are used to construct the *A* and *B* scales of Figure 8

one can be profitably added to this chart for multiplying total stand values by percentages to get partial stand values (*A* and *A'*, fig. 6). These will be ordinary logarithmic scales with cycles matched with those of the central scale.

Stand tables may also be included by using a series of percentage scales, but a more usable and simpler way of presenting them is by means of the stand-table graph employing "frequency" paper where such paper fits the data. In a number of cases the arithmetic paper specifically described by Bruce has failed to represent the data by straight lines. With one species the logarithmic type was resorted to in order to straighten the frequency curves. For another species neither type of paper gave satisfactory results at first. Straight

lines were finally obtained by plotting the percentages on logarithmic paper over the diameter *plus a constant*. This constant was the same for stands of any average diameter. For still another species the constant added varied with the average diameter of the stand, the

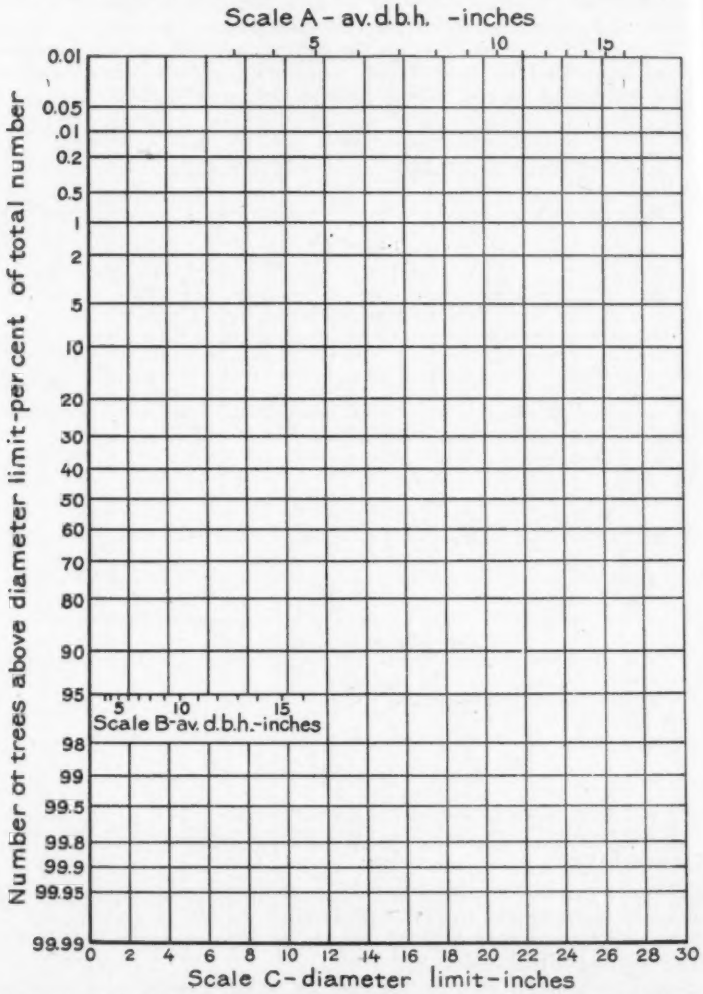

FIG. 8.—Stand-table chart. The frequency distribution in a stand will be shown by a straight line passing through the *A* and *B* scales at the average diameter (by basal area) of the stand

best results being obtained when the constant added for each stand was the same as its average diameter.

The pair of charts used by Bruce for presenting the stand tables graphically can be simplified and combined in one chart, easier to use, by substituting two additional scales to take the place of his

two curves of standard deviation and average diameter. To do this, two somewhat similar curves are prepared from the readings of the basic frequency curves [10] at percentages as far apart on the chart as convenient—in the example given here the readings of 95 and 0.01 per cent are used. The diameters at which each frequency curve intercepts the two percentage lines selected are plotted over the average diameter for this frequency curve. A curve is fitted to each of the two series of points, as shown in Figure 7. Reading back from these two curves, two scales of average diameter are constructed along the percentage lines. These are scales *A* and *B* in Figure 8. To locate the line representing the frequency distribution in a stand of given average diameter all that is necessary is to draw a line passing through this average diameter on both of the *A* and *B* scales.

It will be noted that the frequency graph presented here has percentages along the vertical scale and diameters along the horizontal scale, in keeping with the standard practice of using abscissas for the independent variable and ordinates for the dependent variable. The frequency paper as printed does not conform to this standard.

SUMMARY

The modification here reported of Bruce's method of preparing timber-yield tables consists, chiefly, in expressing the conventional system of curves by means of alignment charts. The curves developed by Bruce's method may be converted into alignment-chart form, but several advantages accrue from deriving the alignment charts directly from the basic data. Chief among these advantages are the reduction in the number of curves to be fitted, with a consequent increase in definition of each curve, and the saving in labor made possible by the ease of interpolating the alignment charts for any age and site index. In addition to the saving of labor in preparing the yield tables the alignment-chart form permits the presentation of final results in greatly condensed form; a single sheet of pocket notebook size is sufficient to carry the results with enough accuracy for field use.

To insure greater accuracy an additional cross check between height, stand basal area, cubic volume, and "forest form factor" has been incorporated. Several minor errors in Bruce's method have been corrected. A simpler method of computing and graphically presenting stand tables has been described.

The method, as modified, has no disadvantages not inherent in Bruce's method, except that partial-stand values can not be read directly but must be obtained through converting factors. It has all the advantages of Bruce's method plus the advantages resulting from the use of alignment charts.

[10] The basic curves should be prepared from the data grouped by average-diameter classes, instead of by age-site index classes as used by Bruce. No variation in form of the curves has been detected between stands of the same average diameter but of different age or site quality. The age-site index grouping was desirable, in technic development, for analysis. Since the soundness of the technic has been established, however, it is more desirable to use the grouping by average diameter, with its resultant stronger, fewer curves.

APPENDIX

INSTRUCTIONS FOR USING ALIGNMENT CHART YIELD TABLE

For—	Hold age on—	Hold SI on—	Read—	Multiply by—
A. For site classification hold age on *B*, hold height of average dominant on *X*, and read site index on *B'*.				
B. Height of average dominant	B	B'	X	
C. Entire stand:				
1. Average d. b. h., inches	C	C'	X'	
2. Tree basal area, square feet	C	C'	X	0.01
3. Number of trees per acre	D	D'	X	10
4. Basal area, square feet per acre	E	E'	X	
5. Volume, total, less bark, cubic feet per acre	F	F'	X	100
D. Partial stand: For any of the five factors listed under *C* determine its entire-stand value first. Determine average d. b. h. (*C*–1) also. Pass a line through this average d. b. h. (on *X'*) and the point *P'*.				
1. For the stand 4 inches plus—				
Read average d. b. h. on	L			
Read per cent number of trees on	N			
Read per cent volume (cubic feet) on	O			
Read cubic feet per cord on	M			
2. For the stand 7 inches plus—				
Read average d. b. h. on	I			
Read per cent number of trees on	G			
Read board feet per cubic foot on	H			
3. For the dominant stand—				
Read average d. b. h. on	J			
Read per cent number of trees on	K			

Multiply the entire-stand values by the percentages and ratios read, holding the entire-stand value on *A*, the percentage or ration on *A'*, reading the partial-stand value on *X* pointing off as with a slide rule.

NOTES.—The cubic feet per cord values (*M*) represent the ratio of cubic feet (entire stem, less bark) to cords (to 3-inch top d. i. b.)

The board feet per cubic foot values (*H*) represent the ratio

$$\frac{\text{Board feet stand 7-inch plus}}{\text{Total cubic feet entire stand.}}$$

THE SUPPLEMENTARY RELATION BETWEEN THE PROTEINS OF CORN AND OF TANKAGE DETERMINED BY METABOLISM EXPERIMENTS ON SWINE[1]

By H. H. MITCHELL and C. H. KICK, *Division of Animal Nutrition, Illinois Agricultural Experiment Station*

INTRODUCTION

High-grade tankage, or meat meal, is one of the most effective supplements to corn in the growing and fattening of swine. The tankage supplements the corn (1) by contributing calcium to a feed that is exceptionally low in this essential mineral, and (2) by supplying a large quantity of protein to a feed comparatively deficient in this material.

This paper is concerned with the protein relation between corn and tankage, and particularly with the question whether the effect of tankage proteins in this combination is due only to their intrinsic value in nutrition, or whether it is also due to a supplementary relation with the proteins of corn, by which each source of protein increases the utilization of the other in anabolism. The problem evidently requires the biological evaluation of the proteins of corn and tankage alone and in combination by the use of quantitative methods.

For reasons that have been discussed fully elsewhere (*8*),[2] it appears that the most direct method of studying the biological efficiency of the proteins from different food materials is the nitrogen metabolism experiment, in which the wastage of dietary nitrogen in digestion and metabolism can be determined from the nitrogen excretion in feces and urine as compared with the excretions on a nonnitrogenous diet. It is true that the values thus obtained relate to the total nitrogen or the crude protein of the food material rather than to the true protein; however, it appears that the nonprotein nitrogenous constituents of foods should not be disregarded in determining their protein values (*12*), even though it were possible to do so in such experimental investigations.

PREVIOUS INVESTIGATIONS

A number of investigations of the nitrogen metabolism of growing pigs receiving protein from individual feeds or from definite combinations of feeds have been reported by McCollum (*6*) and by Hart and Steenbock (*3*) from the Wisconsin Agricultural Experiment Station. In these investigations the biological efficiency of the protein fed is measured by the nitrogen balance, expressed either as a percentage of the nitrogen intake, or as a percentage of the absorbed nitrogen, determined in the usual way. While these measures may possess a good deal of practical significance, their scientific value is impaired by the fact that the nitrogen balance does not represent the total retention of nitrogen from the nitrogen consumed or absorbed,

[1] Received for publication Aug. 4, 1927; issued December, 1927.
[2] Reference is made by number (italics) to "Literature cited," p. 863.

since it neglects the nitrogen used for maintenance. In replacing nitrogen losses from the body occurring as a result of the endogenous katabolism, dietary nitrogen is serving in a substitutive way, as contrasted with its additive utilization in growth, represented by the nitrogen balance. A comparison of the intake of nitrogen with the nitrogen added to the body neglects the nitrogen that is replacing body nitrogen, and hence is crediting the intake with only part of its actual usefulness in covering the protein requirements of the animal.

These measures of biological efficiency are, therefore, too low; they will vary not only with the quality of protein fed, but also with the amount consumed. The greater the consumption of a given protein the greater the proportion of it that will be used for growth and the more nearly will the nitrogen balance represent its total value to the body, and consequently the higher will the percentage "retention" become.

This objection to such measures of protein efficiency is overcome by measuring what has been called by Thomas (*15*) the "biological value" of the dietary protein (nitrogen). The biological value is the percentage of the absorbed nitrogen that is retained for both maintenance and growth, and hence is not eliminated in the urine. Its calculation involves indirect estimations of the food nitrogen in both feces and urine, as distinguished from excreted body nitrogen. Methods by which these estimations may be made and the assumptions necessarily involved have been discussed by one of the present authors (*9*). These methods, originally used in metabolism experiments on rats, have been successfully applied to pigs in the experiments to be described in this paper.

The metabolism experiments of McCollum and of Hart and Steenbock may be recomputed so as to yield estimates of the biological values of the proteins fed by assuming average values of the endogenous losses of nitrogen by pigs (*8*). In McCollum's experiments (*6*) the endogenous losses of nitrogen in the urine may be estimated from the observed excretions of creatinine nitrogen on the basis of earlier observations of this investigator (*7*) to the effect that the total endogenous nitrogen in the pig averages 5.5 times the creatinine nitrogen. These calculations give average biological values of 48 for corn (5 pigs) compared with 44 for wheat (4 pigs), 42 for oats (4 pigs), 67 for casein (1 pig), and 80 for milk (1 pig) on rations containing from 7 to 17 per cent of protein.

Experiments by Hart and Steenbock (*3*) on swine, recalculated in a less satisfactory way (*8*), have indicated a biological value of 60 for corn proteins. In a number of experiments on rats one of the writers has obtained (*10*) an average biological value of 60 for corn proteins at a 10 per cent level of intake.

The only experiments of which the writers are aware relating to the biological value of tankage were published from this laboratory over three years ago (*10*). In six experiments upon three rats an average biological value of 31 was obtained on a ration containing 10 per cent of tankage protein. That a low biological value may be characteristic of the proteins of several animal by-products is also indicated by other experiments of Hart and Steenbock on swine (*2*). Recalculating the results of these experiments on the assumption of average endogenous losses of nitrogen, an average biological value of

49 was obtained for meat crisps and 58 for fish meal from three pigs in each case.

A combination of corn proteins and tankage proteins in equal parts has been shown to have an average biological value of 62 for rats (*11*), somewhat higher than the value for corn alone at the same level of intake, i. e., 10 per cent. The experiments of Hart and Steenbock (*2*) on pigs indicate a biological value of 68 for a mixture of corn and tankage proteins in the proportion of approximately 3 to 2.

From the results on rats it appears that the proteins of tankage are of exceptionally low biological value, but that when combined with corn proteins such a marked supplementary relation exists that the mixture possesses a slightly higher biological value than the proteins of corn. The data available on swine, such as they are, tend to support this conclusion, but they are not sufficiently exact to demonstrate it. Hence an experiment on pigs, carried out in essentially the same manner as those previously reported on rats, has been undertaken with the results described below.

METHODS

Eight young Poland-China barrows were used in this investigation. They were confined in metabolism crates very similar in construction to that described by Forbes (*1*), the only essential difference consisting in the substitution of a fine copper screen for the cloth used to retain the feces. Collections of feces and urine were made daily, and the length of collection periods in all cases, except where otherwise indicated in the tables, was 10 days.

The urine and washings from the crates were aliquoted daily and composited for analysis. The feces were collected, dried at temperatures of 65° C. or less, ground finely, and analyzed. Nitrogen determinations on feed, feces, and urine were made according to the Gunning-Arnold-Dyer modification of the Kjeldahl method.

The pigs were first placed on a nitrogen-free ration consisting of a high-grade cornstarch 96 per cent and a mineral mixture 4 per cent. The mineral mixture used contained steamed bone meal 30 parts, ground limestone 30 parts, sodium chloride 30 parts, magnesium carbonate 3 parts, potassium carbonate 3 parts, potassium sulphate 2 parts, ferric chloride 1.5 parts, and potassium iodide 0.5 part.

After 12 to 14 days on this ration it was found that the output of urinary nitrogen had reached a level, and the first standardizing period was started at this time, using the starch and salts ration.

The second experimental period involved the feeding of corn, the third period the feeding of corn and tankage in the proportion of 2 parts of corn nitrogen to 1 part of tankage nitrogen, the fourth period the feeding of tankage alone, while the fifth period was a final standardizing period on starch and salts. All of these rations contained 4 per cent of the mineral mixture described above and either 2 or, in the later experiments, 5 per cent of cod-liver oil. The amount of protein-containing foods and of starch used was adjusted so that the ration would contain approximately 8 to 10 per cent of crude protein, but this result was not attained in all cases; the nitrogen content of the rations exclusive of the cod-liver oil, which was added when the rations were offered to the pigs, was as shown in Table 1.

TABLE 1.—*Nitrogen content of the experimental rations* [a]

Pig Nos.	Nitrogen content of—		
	Corn	Corn and tankage	Tankage
	Per cent	*Per cent*	*Per cent*
1, 2, 3	1.28	1.06	1.25
4, 5, 6, 7, 8	1.39	1.49	1.62

[a] These rations were analyzed before the addition of the cod-liver oil. The cod-liver oil was added to the feed just before it was given to the pigs.

A good grade of yellow corn and guaranteed 60 per cent tankage were used in these experiments. The experimental periods were separated by 7 to 10 days of preliminary feeding, in which the pigs received the same rations in the same amounts as were fed in the following collection period.

EXPERIMENTAL RESULTS

The results of these nitrogen balance studies are assembled in Table 2, together with the biological values computed from them and the more significant intermediate values.

The biological values calculated for the different rations presumably represent the percentages of the absorbed nitrogen used by growing pigs for both maintenance and growth. Their calculation involves the assumption that the excretion of fecal nitrogen per kilogram of food consumed on the nitrogen-free ration in the first and final periods measures the excretion of body nitrogen in the feces in the intervening periods.[3] The change in the excretion of the fecal nitrogen per kilogram of nitrogen-free ration from the first to the fifth period is assumed to occur in a linear fashion with respect to time. The second assumption involved in these calculations is that the excretion of nitrogen in the urine per kilogram of body weight in the first and final periods of nitrogen-free feeding is a measure of the excretion of body nitrogen in the urine in the intervening periods, the change in these values from the first to the fifth period being also assumed to be linear.

For convenience of study, the biological values obtained from the nitrogen metabolism experiments have been collected in Table 3, together with computations of the true digestibility of the dietary protein, allowance being made for the metabolic fecal nitrogen.

[3] In a recent paper appearing in this Journal (*16*), H. W. Titus concludes that "the fecal nitrogen excretion of a steer consuming a nitrogen-free ration can not safely be taken as a measure of the metabolic nitrogen in the feces of the animal when consuming an equal weight of a given feeding stuff." It appears to the present writers, however, that this conclusion is based upon a debatable mathematical interpretation of data obtained on nitrogen-containing rations. Equation (3) of this article seems contradictory to the conclusion stated, and yet it is used in the argument upon which the conclusion is based. From the fact that the total fecal nitrogen in his experiments appeared to be positively correlated with the moisture content of the feces, Titus concludes that "the amount of metabolic nitrogen in the feces of a steer is influenced, among other things, by the water content of the feces," without disposing of the equally plausible alternative explanation that the digestibility of the feed nitrogen is so influenced. Thus, it is quite possible that equation (2) would be more accurate if it did not contain the b term; in which case, again, the conclusion of Titus first quoted would become untenable. Therefore, there appears to be nothing in the data or arguments that Titus has offered which necessarily contradicts the position taken in this and other papers from this laboratory, that the metabolic nitrogen in the feces from a given ration may be satisfactorily estimated from the fecal nitrogen produced on an equal amount of nitrogen-free dry matter of approximately the same crude-fiber content.

TABLE 2.—*Nitrogen metabolism data and the calculation of biological values*

PERIOD 1. STARCH RATION

Animal No.	Initial weight	Final weight	Food intake	Nitrogen intake	Fecal nitrogen	Metabolic nitrogen in feces	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value
	Kgm.	*Kgm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Pct.*
1	35.2	35.4	520	------	0.58	[a]1.12	------	------	1.81	[b]0.051	------	------	------
2	35.2	36.5	520	------	.43	[a].83	------	------	1.80	[b].050	------	------	------
3	39.0	39.0	520	------	.45	[a].87	------	------	2.07	[b].053	------	------	------
4[c]	56.2	52.6	1,000	------	.72	[a].72	------	------	4.39	[b].081	------	------	------
5[c]	50.3	50.8	1,000	------	1.04	[a]1.04	------	------	2.71	[b].054	------	------	------
6[d]	48.1	45.4	1,000	------	.54	[a].54	------	------	3.47	[b].074	------	------	------
7[c]	43.1	43.5	1,000	------	.75	[a].75	------	------	2.48	[b].057	------	------	------
8[c]	50.3	49.9	1,000	------	.90	[a].90	------	------	3.75	[b].075	------	------	------

PERIOD 2. CORN RATION

1	37.6	37.4	600	7.68	2.16	0.62	1.54	6.14	4.55	1.95	2.60	3.54	58
2	37.6	37.4	600	7.68	1.79	.55	1.24	6.44	5.08	1.87	3.21	3.23	50
3	40.8	40.6	600	7.68	2.01	.56	1.45	6.23	4.84	2.08	2.76	3.47	56
4	57.6	58.1	1,000	13.62	2.51	.77	1.74	11.88	8.82	4.27	4.55	7.33	62
5	52.2	54.0	1,000	13.62	3.03	1.00	2.03	11.59	8.85	2.82	6.03	5.56	48
6	49.9	51.3	1,000	13.62	2.32	.62	1.70	11.92	8.91	3.31	5.60	6.32	53
7	45.8	46.7	1,000	13.62	2.61	.93	1.68	11.94	8.60	2.85	5.75	6.19	52
8	51.7	52.6	1,000	13.62	2.76	.88	1.88	11.74	8.85	3.46	5.39	6.35	54

PERIOD 3. CORN AND TANKAGE RATION

1	38.1	38.6	700	7.29	1.72	0.66	1.06	6.23	4.35	2.01	2.34	3.89	62
2	38.6	39.0	700	7.29	1.39	.71	.68	6.61	4.35	1.92	2.43	4.18	63
3	41.3	43.8	700	7.29	1.63	.69	.94	6.35	4.70	2.08	2.62	3.73	59
4	60.1	61.2	1,000	14.60	4.67	.82	3.85	10.75	7.94	4.06	3.88	6.87	64
5	56.0	57.4	1,000	14.60	3.97	.96	3.01	11.59	7.81	3.00	4.81	6.78	58
6	53.1	55.6	1,000	14.60	2.97	.70	2.27	12.33	7.53	3.09	4.44	7.89	64
7	48.5	50.8	1,000	14.60	4.51	1.11	3.40	11.20	7.74	3.28	4.46	6.74	60
8	54.2	56.2	1,000	14.60	4.26	.85	3.41	11.19	8.24	3.19	5.05	6.14	55

PERIOD 4. TANKAGE RATION

1	38.6	39.7	700	8.61	1.94	0.60	1.34	7.27	5.84	2.08	3.76	3.51	48
2	38.6	39.7	700	8.61	1.87	.76	1.11	7.50	5.88	1.91	3.97	3.53	47
3	41.7	41.1	700	8.61	2.21	.74	1.47	7.14	6.90	1.93	4.97	2.17	30
4	64.4	65.3	1,000	15.89	5.69	.87	4.82	11.07	9.65	3.88	5.77	5.30	48
5	59.4	59.9	893	14.19	5.04	.81	4.23	9.96	8.95	3.13	5.82	4.14	42
6	57.2	58.5	1,000	15.89	5.16	.78	4.38	11.51	10.40	2.79	7.61	3.90	34
7	53.1	55.3	1,000	15.89	4.84	1.29	3.55	12.34	9.46	3.81	5.65	6.69	54
8	56.9	59.2	1,000	15.89	6.62	.82	5.80	10.09	9.70	2.86	6.84	3.25	32

PERIOD 5. STARCH RATION

1	40.1	41.5	700	------	0.54	[a]0.77	------	------	2.19	[b]0.054	------	------	------
2	39.9	40.8	700	------	.82	[a]1.17	------	------	1.96	[b].048	------	------	------
3	40.8	41.3	700	------	.77	[a]1.10	------	------	1.83	[b].045	------	------	------
4[c]	66.5	69.6	1,000	------	.92	[a].92	------	------	3.60	[b].053	------	------	------
5	61.7	64.4	1,000	------	.87	[a].87	------	------	3.29	[b].052	------	------	------
6	59.2	61.2	1,000	------	.82	[a].82	------	------	2.84	[b].039	------	------	------
7[c]	54.7	55.6	1,000	------	1.47	[a]1.47	------	------	4.12	[b].075	------	------	------
8	60.3	61.2	1,000	------	.80	[a].80	------	------	2.48	[b].041	------	------	------

[a] Fecal nitrogen per kilogram of dry feed. These values were used in estimating the metabolic nitrogen in the feces in periods 2, 3, and 4. The change in the ratio of metabolic nitrogen to dry matter consumed from period 1 to period 5 was assumed to occur in a linear fashion.

[b] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous (body) nitrogen in the urine in periods 2, 3, and 4, the same assumption of a linear variation from period 1 to period 5 being made as in the case of the metabolic nitrogen in the feces.

[c] This is a 7-day collection period instead of a 10-day period.

[d] This is a 4-day collection period.

[e] This is an 8-day collection period.

TABLE 3.—*A summary of the coefficients of digestibility and the biological values of protein*

Pig No.	Coefficients of digestibility of protein.*			Biological values of protein		
	Corn	Corn and tankage	Tankage	Corn	Corn and tankage	Tankage
1	80	85	84	58	62	48
2	84	91	87	50	63	47
3	81	87	83	56	59	30
4	87	74	70	62	64	48
5	85	79	70	48	58	42
6	87	84	72	53	64	34
7	88	78	78	52	60	54
8	86	77	63	54	55	32
Average	85	82	76	54	61	42

* Corrected for metabolic nitrogen in the feces.

The experiments on pigs 1, 2, and 3 preceded the experiments on pigs 4, 5, 6, 7, and 8 by about one year.[4] Some of the differences in the results obtained with these two groups of animals, particularly with reference to digestibility of the protein, may therefore be related to differences in the lots of feed used.

The digestion coefficients were obtained from the estimates of absorbed nitrogen given in Table 2, which involve the assumption that the excretion of total fecal nitrogen in periods 1 and 5, when the pigs were on nitrogen-free rations, measures the excretion of metabolic nitrogen in the feces on equal amounts of protein-containing rations. The justification for this assumption has been discussed at some length by one of the writers in another publication (*12*).

In these trials the average true digestibility of the nitrogen of corn was 85 per cent. The coefficients for the last five pigs were appreciably higher than those for the first three pigs. The tankage used with the group of five pigs was evidently much less digestible than that used for the group of three pigs. This difference is reflected in the coefficients obtained with the combination of corn and tankage.

The biological values, however, do not show these group differences, although considerable variation among individual results is evident for each ration. The average value for corn protein alone was 54, for tankage protein alone 42, and for corn and tankage proteins in the proportion of 2 to 1, 61. The average results thus confirm the general conclusions of the rat-feeding experiments previously published (*11*) to the effect that tankage protein (nitrogen) is of lower biological value than the protein of corn, but that, when combined with corn proteins, a protein mixture superior in quality to that of corn is obtained.

Each of the eight pigs, with one exception, gave the lowest biological value for tankage proteins, an intermediate value for corn proteins, and the highest value for the combined proteins. The exceptional pig, No. 7, would fall in line with the others if the exceptionally high biological value for tankage protein could reasonably be considered in error. In looking over the data for this pig for period 4 on tankage

[4] The experiments on pigs 1, 2, and 3 were run from Sept. 24 to Dec. 23, 1925; those on the remaining pigs were run from Nov. 1, 1926, to Feb. 10, 1927.

feeding, the urinary nitrogen is not exceptionally low, but the absorbed nitrogen appears to be estimated too high, due to an abnormally high metabolic nitrogen factor obtained in the following period. The individual data may reasonably be considered, therefore, as substantiating unanimously the conclusion indicated by the average biological values for the proteins of the three experimental rations.

The low biological value of tankage proteins (nitrogen) is not a matter of surprise when it is considered that much of the protein material of which tankage is composed if of low biological value. The bone in tankage, as well as much of the soft tissues, carries albuminoid proteins (particularly collagen) of low nutritive value, and the blood protein, which is commonly added for the purpose of adjusting the nitrogen content to the guaranteed value, must also be classed as of low biological value. Steck (*14*) has obtained very definite indications that hemoglobin, constituting almost two-thirds of the protein of blood, is poorly utilized in anabolism, while Hoagland and Snider (*4*) have not obtained economical growth in rats on blood serum proteins alone.

Nor is the marked supplementary relation between tankage and corn proteins quite unexpected. Animal tissue proteins have been shown to possess a high supplemental value for cereal proteins by one of the writers (*13*) and, more extensively, by Hoagland and Snider (*5*).

SUMMARY

In nitrogen metabolism studies on eight growing pigs, the true digestibility and the biological value of the proteins (nitrogen) of corn and of tankage, as well as of a mixture of these in the ratio of 2 to 1, were determined by methods that have heretofore been applied to rats only.

The average coefficients of digestibility of protein, corrected for the metabolic nitrogen of the feces, were 85 for corn, 76 for tankage, and 82 for the mixture. Apparently different samples of tankage may differ greatly in this respect.

The average biological values for protein (nitrogen) were 54 for corn, 42 for tankage, and 61 for the combination. The individual pigs, with one doubtful exception, gave values showing the same relations as the average values.

The crude protein of tankage evidently is of low nutritive value in covering the nitrogen requirements of growing swine as well as of growing rats. When combined with corn protein, however, a marked supplementary relation is demonstrable; so that the biological value of the mixed proteins is slightly higher than that of corn alone.

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